UNIVERSIDADE FEDERAL DO PARANÁ

VIVIAN JASKIW SZILAGYI ZECCHIN

USO DA BACTÉRIA PROMOTORA DO CRESCIMENTO VEGETAL, *Bacillus amyloliquefaciens* SUBSP. *plantarum* FZB42, NO TOMATEIRO EM CULTIVO ORGÂNICO

Curitiba-PR

2016

VIVIAN JASKIW SZILAGYI ZECCHIN

USO DA BACTÉRIA PROMOTORA DO CRESCIMENTO VEGETAL*, Bacillus amyloliquefaciens* SUBSP. *plantarum* FZB42, NO TOMATEIRO EM CULTIVO ORGÂNICO

Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanitarismo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Doutora em Ciências.

Orientador: Prof. Dr. Átila Francisco Mógor

Co-orientadora: Profª. Dra. Lucimeris Ruaro

Curitiba-PR

2016

Z42 Zecchin, Vivan Jaskiw Szilagyi
Uso da bactéria promotora do crescimento vegetal, *Bacillus amyloliquefaciens* SUBSP. *plantarum* FZB42, no tomateiro em cultivo orgânico. / Curitiba: 2016.
93 f. il.

Orientador: Átila Francisco Mógor
Tese (Doutorado) – Universidade Federal do Paraná.
Setor de Ciências Agrárias. Programa de Pós-Graduação em Agronomia.

1. Tomate – Cultivo. 2. Biofertilizantes. 3. Agricultura orgânica. I. Mógor, Átila Francisco. II. Universidade Federal do Paraná. Setor de Ciências Agrárias. Programa de Pós-Graduação em Agronomia. III. Título.

CDU 635.64

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM
AGRONOMIA - PRODUÇÃO VEGETAL

PARECER

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Agronomia - Produção Vegetal, reuniram-se para realizar a argüição da Tese de DOUTORADO, apresentada pela candidata **VIVIAN JASKIW SZILAGYI ZECCHIN**, sob o título **"USO DA BACTÉRIA PROMOTORA DO CRESCIMENTO VEGETAL *Bacillus amyloliquefaciens* SUBSP. *plantarum* FZB42 NO TOMATEIRO EM CULTIVO ORGÂNICO"**, para obtenção do grau de Doutor em Ciências do Programa de Pós-Graduação em Agronomia - Produção Vegetal do Setor de Ciências Agrárias da Universidade Federal do Paraná.

Após haver analisado o referido trabalho e argüido a candidata são de parecer pela "**APROVAÇÃO**" da Tese.

Curitiba, 14 de junho de 2016.

Professor Dr. Cícero Deschamps
Coordenador do Programa

Dra. Gilda Mógor
Primeira Examinadora

Professora Dra. Lygia Vitória Galli Terasawa
Segunda Examinadora

Professor Dr. João Carlos Bespalhok Filho
Terceiro Examinador

Professor Dr. Cícero Deschamps
Quarto Examinador

Professora Dra. Lucimeris Ruaro
Quinta Examinadora

Professor Dr. Átila Francisco Mógor
Presidente da Banca e Orientador

Esta tese é dedicada às minhas filhas, Maria Clara e Luisa, que me trouxeram alegria desde o dia que se fizeram presentes em minha vida.

AGRADECIMENTOS

A Deus, por olhar por mim me concedendo saúde e força para percorrer esta caminhada superando as dificuldades.

Aos meus pais Maria Doracy Jaskiw e Luiz Szilagyi Filho, que me apoiaram e incentivaram nessa trajetória. Principalmente à minha mãe que esteve comigo em muitos momentos auxiliando na condução dos trabalhos de forma direta e indireta.

Ao meu marido André Luís dos Santos Zecchin, por me auxiliar tecnicamente nos trabalhos, pelo companheirismo nos momentos de alegrias e dificuldades, inclusive em dias exaustivos de esgotamento físico e mental, e por sempre torcer pelo meu sucesso.

Ao meu orientador, Dr. Átila Francisco Mógor, quem muito admiro, não só pelo conhecimento que possui mas por sua postura frente à vida, por sua contribuição neste trabalho, pela compreensão, e por me incentivar na busca de novas oportunidades.

A minha co-orientadora, Dra. Lucimeris Ruaro, que sempre esteve à disposição de forma amigável.

A toda equipe do grupo de Olericultura Orgânica, em especial à Gilda Mógor, pela atenção e pelo suporte na execução dos trabalhos de laboratório; à Cinthia Röder, Luiz Gabriel Gemim, Juliana Amatussi e Vanessa Orbutz, que me acompanharam em vários experimentos de laboratório e inclusive colheitas de experimentos de campo; à Rodrigo Teles pelo auxílio nas análises morfométricas intermináveis.

Aos membros da minha banca de qualificação e de pré-defesa, Dr. João Bespalhok Filho, Dra. Lucimeris Ruaro, Dra. Lygia Vitória Galli Terasawa, Dr. Cícero Deschamps, Dra. Mariangela Hungria, pelas contribuições para o aprimoramento da tese.

Aos amigos e colegas que fiz durante o doutorado, Jeidi, Christian, Guilherme, Tales, Roger, Micheli, Caterine, que com certeza, contribuiram para tornar este percurso mais agradável e menos árduo.

Aos professores e funcionários da Pós-Graduação, em especial à Lucimara Antunes, Maria Emíla Kudla e Seu Marcos que sempre me auxiliaram no que precisei.

À CAPES, pela concessão de bolsa de estudos e à Universidade Federal do Paraná e ao Programa de Pós-Graduação em Agronomia Produção Vegetal, pela oportunidade de realização do Doutorado.

E a todos que de alguma forma contribuíram para a realização deste trabalho, acreditaram em mim e torceram pelo meu sucesso.

“Nunca ande pelo caminho traçado, pois ele conduz somente até onde os outros já foram”.

Alexander Graham Bell

RESUMO

O tomateiro é uma cultura amplamente difundida, seus frutos podem ser utilizados *in natura* ou industrializados. Portanto possui relevância econômica e importância social pois seus derivados passam por uma extensa cadeia produtiva que gera inúmeros empregos. Atualmente com uma maior conscientização ambiental e também visando a saúde, o uso de tecnologias biológicas que incrementem a produtividade sem aditivos químicos, são alternativas desejáveis. Neste contexto o uso de bactérias promotoras de crescimento vegetal vem de encontro a estas necessidades. A bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, que já tem caracterizada algumas habilidades de interesse agronômico, foi o alvo utilizado no estudo da interação planta x bactéria. Sendo assim, os objetivos deste estudo foram: (i) investigar na bactéria características relacionadas à promoção do crescimento vegetal; (ii) verificar alterações morfométricas das plantas de tomateiro inoculadas com FZB42 na germinação de sementes, na produção de mudas e no crescimento das plantas até 60 dias em sistema orgânico em diferentes doses ($1{,}5x10^{9}$; $6{,}0x10^{10}$; $2{,}4x10^{11}$ bactérias mL^{-1}); (iii) analisar alterações bioquímicas e nutricionais em plantas de tomateiro; (iv) avaliar a produção de tomates mediante inoculação da bactéria nas doses ($1{,}5x10^{9}$; $6{,}0x10^{10}$). FZB42 apresentou resultados positivos para produção de sideróforos e compostos indólicos com e sem suplementação de triptofano. Sementes inoculadas não tiverem seu percentual de germinação alterado. Na produção de mudas, a dose de $6{,}0x10^{10}$, foi a mais estimuladora do crescimento vegetal, com incrementos na parte aérea das cultivares Cereja 261, Santa Clara I-5300, Santa Cruz Kada Gigante e Serato F1, bem como aumentou os teores de clorofila. As mudas das duas últimas cultivares, na mesma dose, também tiveram o sistema radicular estimulado e os teores de açúcares solúveis e proteínas solúveis aumentados. A dose de $1{,}5x10^{9}$ reduziu o crescimento das mudas. Plantas com 60 dias após semeadura, de 'Cereja 261', 'Santa Clara I-5300' e 'Serato F1' apresentaram-se com maior parte aérea e raízes quando inoculadas com $6{,}0x10^{10}$. Plantas de 60 dias submetidas a $1{,}5x10^{9}$ tiveram estímulos mais discretos e quando inoculadas com $2{,}4x10^{11}$ os aspectos biométricos foram diminuídos. Nesta etapa o perfil metabólico dos cultivares não seguiu um padrão, pois estavam sujeitos às variação morfométricas devido às diferenças de estadio fenológico. O cultivar Serato F1 foi conduzido à campo em cultivo protegido. Aos 135 dias após plantio (em plena frutificação) apresentou, nas folhas, açúcares solúveis totais e aminoácidos livres totais em maiores quantidades nas plantas inoculadas com as doses de $1{,}5x10^{9}$ e $6{,}0x10^{10}$. Já os frutos maduros das plantas inoculadas, também colhidos no mesmo momento, apresentaram mais proteínas solúveis totais e aminoácidos livres totais. Neste mesmo período a análise de macro e micro nutrientes revelou que plantas inoculadas tinham maiores teores de nitrogênio, ferro e manganês. A dose de $6{,}0x10^{10}$ diminuiu os níveis de cobre e aumentou zinco. Quanto aos aspectos produtivos, todos os tratamentos com plantas inoculadas apresentaram cerca de quarto frutos a mais por planta, sendo estes frutos de maior calibre e mais pesados que o controle não inoculado. Isso refletiu em uma produção de cerca de 1 kg a mais por planta, o que considerando 12,820 plantas ha^{-1}, representa um incremento de 11,76 e 13,23 ton ha^{-1} nas doses de $1{,}5x10^{9}$ e $6{,}0x10^{10}$, respectivamente. Portanto, FZB42 na dose de $6{,}0x10^{10}$ foi mais adequado para estímulo ao crescimento vegetal na fase de produção de mudas. Em termos de produtividade ambas as doses $1{,}5x10^{9}$ e $6{,}0x10^{10}$ foram eficazes. Sendo assim, FZB42 na dose de $6{,}0x10^{10}$ bactérias mL^{-1} foi efetivo na promoção de crescimento durante todo o ciclo do tomateiro e com isso demonstrou potencial como inoculante ou bio-fertilizantes proporcionando alterações metabólicas e nutricionais.

Palavras-chave: Bactérias promotoras de crescimento vegetal. Inoculante. Bio-fertilizante. Produtividade. *Bacillus amyloliquefaciens*. *Solanum lycopersicum*.

ABSTRACT

The tomato is a widespread crop, its fruits can be used as a raw material for processed food. Therefore it has economic relevance and social importance because is the basis of an extensive production chain that generates numerous jobs. Currently greater environmental awareness and also aimed at health, the use of biological technologies that increase productivity without chemical additives, are desirable alternatives. In this context the use of plant growth promoting bacteria comes to attend these needs. The bacteria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, which already has characterized some desirable agronomically desirable effects, was the aim at this study of plant x bacteria interaction. Thus, the objectives of this study were: (i) investigate the bacteria characteristics related to the promotion of plant growth; (ii) verify morphometric changes of tomato plants inoculated with FZB42 on seed germination, in the production of seedlings, and plant growth to 60 days after sowing in organic system at different doses ($1.5x10^9$; $6.0x10^{10}$; $2.4x10^{11}$ bacteria mL^{-1}); (iii) analyze biochemical and nutritional changes in tomato plants; (iv) evaluate the production of tomatoes by inoculation of bacteria in doses ($1.5x10^9$; $1,6x10^5$). FZB42 tested was positive for siderophore production and indolec compounds with and without tryptophan supplementation. Inoculated seeds have not changed their germination percentage. In the production of seedlings, the dose of $1.6x10^5$ was the most stimulating plant growth, with increases in the shoots of 'Cherry 261', 'Santa Clara I-5300', 'Santa Cruz Kada Gigante' and 'Serato F1', as well as increased levels of chlorophyll. The last two cultivars seedlings at the same dose also had their roots stimulated and showed increaes in total soluble sugars and total soluble proteins. The dose of $2.4x10^{11}$ reduced seedling growth. Plants 60 days after sowing, the 'Cherry 261,' 'Santa Clara I-5300' and 'Serato F1' were improved their shoots and roots growth when inoculated with $6.0x10^{10}$. Plants with 60 days inoculates with $1.5x10^9$ had more discrete stimuli and when inoculated with $2.4x10^{11}$ the growth were diminished. At this stage the metabolic profile of cultivars did not follow a pattern because they were subject to the morphometric variation due differences in phenological stages. The cultivar Serato F1 was cultivated in greenhouse. At 135 days after planting (in full fruiting) the leaves presented total soluble sugars and total free amino acids in a larger amount in the plants inoculated with doses of $1.5x10^9$ and $6.0x10^{10}$. In addition, the ripe fruits of inoculated plants, harvested at this time, had more total soluble protein and total free amino acids. In the same period, the analysis of macro and micronutrients on leaves showed that inoculated plants had higher levels of nitrogen, iron and manganese. The dose of $6.0x10^{10}$ decreased copper and increased zinc levels. All inoculated plants showed the increment of around four fruits per plant, which fruits with greater caliber and heavier than uninoculated control. This reflected in an increasing of about 1 kg at more per plant, considering that 12,820 plants ha^{-1}, representing an increase of 11.76 tons ha^{-1} and 13.23 tons ha^{-1} at the doses of $1.5x10^9$ and $6.0x10^{10}$ respectively. Therefore, *Bacillus amyloliquefaciens* FZB42 at $6.0x10^{10}$ dose was more efficient for stimulating plant growth in seedling production. In terms of yield, both $1.5x10^9$ and $6.0x10^{10}$, doses were effective. Thus, FZB42 at a dose of $6.0x10^{10}$ bacteria mL^{-1} was effective in promoting growth throughout the tomato cycle and it showed potential as inoculant or bio-fertilizer providing nutritional and metabolic changes.

Key-words: Plant growth promoting bacteria. Inoculation. Biofertilizers. Productivity. *Bacillus amyloliquefaciens*. *Solanum lycopersicum.*

LISTA DE FIGURAS

3 CAPÍTULO I - CRESCIMENTO DE MUDAS DE TOMATEIRO (*Solanum lycopersicum*) **ESTIMULADO PELA BACTÉRIA** *Bacillus amyloliquefaciens* **SUBSP.** *plantarum* **FZB42 EM CULTURA ORGÂNICA.**

4 CAPÍTULO II – ALTERATIONS ON METABOLISM OF SUGARS AND PROTEINS AND IN THE PLANT DEVELOPMENT OF TOMATO CULTIVARS BY THE INOCULATION OF DIFFERENT DOSES OF *Bacillus amyloliquefaciens*

5 CAPÍTULO III – GROWTH AND BIOCHEMICAL CHANGES ON TOMATO SEEDLINGS INOCULATED WITH *Bacillus amyloliquefaciens*

6 CAPÍTULO IV – YIELD AND METABOLIC CHANGES ON ORGANICALLY GROWN TOMATO INOCULATED WITH *Bacillus amyloliquefaciens* FZB42

LISTA DE TABELAS

3 CAPÍTULO I - CRESCIMENTO DE MUDAS DE TOMATEIRO (*Solanum lycopersicum*) **ESTIMULADO PELA BACTÉRIA** *Bacillus amyloliquefaciens* **SUBSP.** *plantarum* **FZB42 EM CULTURA ORGÂNICA.**

4 CAPÍTULO II – ALTERATIONS ON METABOLISM OF SUGARS AND PROTEINS AND IN THE PLANT DEVELOPMENT OF TOMATO CULTIVARS BY THE INOCULATION OF DIFFERENT DOSES OF *Bacillus*

5 CAPÍTULO III – GROWTH AND BIOCHEMICAL CHANGES ON TOMATO SEEDLINGS INOCULATED WITH *Bacillus amyloliquefaciens*

6 CAPÍTULO IV – YIELD AND METABOLIC CHANGES ON ORGANICALLY GROWN TOMATO INOCULATED WITH *Bacillus amyloliquefaciens* FZB42

SUMÁRIO

1 INTRODUÇÃO GERAL

O tomate, fruto do tomateiro (*Solanum lycopersicum*) é a primeira hortaliça fruto de importância econômica no Brasil. Esta importância está ligada ao aspecto não só econômico, mas também social e, segundo o levantamento da FAO – Organização das Nações Unidas para Alimentação e Agricultura, o tomate é o décimo primeiro alimento mais produzido mundialmente, contando com aproximadamente 161 milhões de toneladas colhidas em 2012. O Brasil destaca-se com uma produção de aproximadamente 3,8 milhões de toneladas, colocando-se na 9º posição da produção mundial no ano de 2012 (FAO, 2015).

Em um contexto agrícola, a redução do uso de produtos químicos ou o melhor aproveitamento destes dentro do sistema produtivo tem ganhado destaque. Neste caminho, bactérias com habilidades biotecnológicas das vem despertando atenção, por se enquadrarem num modelo de agricultura sustentável, que além de produtividade, também se preocupa com a conservação do meio ambiente (VALE *et al*., 2010). Uma das estratégias consiste em explorar os benefícios da ação dos microrganismos sobre o cultivo de plantas na forma de inoculantes (LUCY *et al*., 2004).

Bactérias do gênero *Bacillus* são gram-positivas e podem ser aeróbias, facultativas ou anaeróbias. São resistentes ao calor e a maioria delas tem exigências nutricionais simples, requerendo no máximo alguns aminoácidos ou vitaminas B como fatores de crescimento (STANIER, 1969). Formam endósporos (estruturas de resistência) e apresentam a habilidade de produzir antibióticos (FREITAS & PIZZINATTO, 1997). A formação de endósporos aumenta a resistência aos fatores adversos. Dessa forma, podem ser armazenados como inoculantes por um período mais longo, e possuem maior tempo de permanência no solo (PETRAS & CASIDA, 1985).

Bacillus amyloliquefaciens FZB42 [Krebs et al. 1998] é a espécie tipo *B. amyloliquefaciens* subsp. *plantarum* (BORRIS *et al*., 2011). Esta cepa tem algumas habilidades agronômicas bem caracterizadas, como a produção de compostos indólicos (SZILAGYI-ZECCHIN *et al.*, 2015a; IDRIS *et al*., 2007), sideróforos (SZILAGYI-ZECCHIN *et al*., 2015a; CHEN *et al*., 2007), fitase (IDRIS *et al*., 2002). Não produz 1-aminociclopropano-1-carboxilase (ACC) deaminase (CUARTAS, 2010), não fixa nitrogênio pois não apresenta em seu genoma genes referentes a essa característica (CHEN *et al*., 2007). Inclusive esta cepa foi a primeira

representante de bactérias promotoras do crescimento de plantas gram-positivas a ter o genoma inteiro sequenciado (CHEN *et al*., 2007).

Pesquisas empregando *Bacillus* com efeitos positivos em termos de promoção do crescimento vegetal vem sendo relatadas: *Bacillus amyloliquefaciens* aumentou a área foliar de cultivares de tomateiro em cerca de 31,6% (SZILAGYI-ZECCHIN *et al*., 2015a); *Bacillus* sp. promoveu aumento no comprimento radicular (65,1%) e na parte aérea (39,4%) de plantas de milho (SZILAGYI-ZECCHIN *et al*., 2015b); *Bacillus* sp. incrementaram e a germinação das sementes de milho em 56% e o volume radicular em 39% (SZILAGYI-ZECCHIN *et al*., 2014).

Além de incrementos no crescimento vegetativo, *Bacillus* são capazes de alterar a produtividade, de diversas espécies de interesse agrícola, como soja (ARAUJO & HUNGRIA, 1999), cebola (HARTHMANN *et al*., 2010), tomate e pimentão (GARCÍA *et al*., 2004).

Na Europa é possível encontrar *B. amyloliquefaciens* FZB42 em produtos comerciais, de pelo menos dois fabricantes diferentes, formulado em pó e líquido e destinado à aplicação no solo ou em sementes. Estes produtos são indicados para uso em olerícolas como batata, cenoura, alface, grandes culturas como milho e algodão e inclusive para plantas ornamentais. Aqui no Brasil, segundo os anexos da Instrução Normativa Nº 13, de 24 de março de 2011 do MAPA, não há produtos autorizado ou recomendado a base de bactérias, seja como inoculante ou biofertilizante para uso em olerícolas, mais especificamente tomate.

De acordo com o Decreto Nº 4.954, de 14 de janeiro de 2004 do Ministro de Estado da Agricultura, Pecuária e Abastecimento (MAPA), conceitualmente inoculante é produto que contém microrganismos com atuação favorável ao crescimento de plantas. E de acordo com a Instrução Normativa Nº 46, de 6 de outubro de 2011, biofertilizante é todo produto, que contém componentes ativos ou agentes biológicos, capaz de atuar, direta ou indiretamente, sobre o todo ou parte das plantas cultivadas, melhorando o desempenho do sistema de produção e que seja isento de substâncias proibidas pela regulamentação de orgânicos. Sendo assim estas duas categorias podem contemplar produtos a base de microrganismos que beneficiem as plantas.

Este estudo fundamenta-se na importância que a cultura do tomateiro representa para o Brasil. Portanto desta maneira, estudos que busquem alternativas para desonerar custos e perdas na produção, por meio da diminuição do uso de insumos químicos, vem colaborar para um sistema agrícola mais sustentável e rentável. Portanto, a avaliação do efeito biofertilizante/inoculante de bactérias é uma importante ferramenta, para o desenvolvimento de estratégias de manejo de culturas e futuras formulações de produtos. Com base no exposto acima, a hipótese deste trabalho pauta-se na concepção de que *B. amyloliquefaciens* subsp.

plantarum FZB42 irá promover o crescimento do tomateiro de maneira crescente de acordo com as doses aplicadas. Sendo assim, o trabalho foi organizado em quatro capítulos que apresentam os seguintes objetivos: Capítulo I - verificar o efeito a bactéria *B. amyloliquefaciens* subsp. *plantarum* FZB42 na germinação e na produção de mudas de dois cultivares conduzidos em sistema orgânico, além de investigar características bacterianas relacionadas à promoção do crescimento vegetal por meio de testes bioquímicos para sideróforos e compostos indólicos; Capítulo II – analisar alterações biométricas e bioquímicas, referentes a açúcares e proteínas, em dois cultivares de tomateiro conduzidos até 60 dias, com sementes inoculadas com a bacteria *Bacillus amyloliquefaciens* subs. *plantarum* FZB42, e identificar seu potencial como inoculante ou biofertilizante; Capítulo III - determinar o crescimento radicular e parte aérea de dois cultivares de tomateiro, em casa de vegetação, cujas sementes foram inoculadas com *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 em diferentes concentrações. Analisar também as alterações bioquímicas relacionadas aos teores de clorofila, carotenoides e açúcares, e ainda nesse capitulo, determinar a capacidade de produção de compostos de indólicos na presença e ausência de triptofano; Capítulo IV – quantificar a produtividade, e analisar alterações metabólicas referentes à açucares, aminoácidos e proteínas em frutos e folhas, além de monitorar o estado nutricional os macro e micro nutrientes das plantas mediante inoculação com a bactéria *Bacillus amyloliquefaciens* subs. *plantarum* FZB42, com a intenção de identificar o seu potencial como inoculante ou bio-fertilizantes.

2 REVISÃO DE LITERATURA

2.1 Bacterial characteristics of agronomic importance

Full text on: SZILAGYI-ZECCHIN, V. J.; MÓGOR, Á. F.; FIGUEIREDO, G. G. O. Strategies for Characterization of Agriculturally Important Bacteria. In: SINGH, D.P.; SINGH, H.B.; PRABHA, R (eds) Microbial Inoculants in Sustainable Agricultural Productivity. Springer, 2016. p. 1-21.

2.1.1 Bio-products, biofertilizer and biopesticides

For facing the challenge to feed humankind on an ecologically friendly way, a new agriculture comes driven by a critical consciousness, knowledge and technology, having on bio-products an effective tool. Biofertilizers are a class that aggregates a range of bio-products related their bioactivity, to improving biological processes.

Biofertilizers and biopesticides hold the potential to increase agricultural productivity with a sustainable approach. A number of countries such as Argentina, Canada, South Africa, India, Australia, the Philippines, United States of America and Brazil, among others, have embraced these technologies (Simiyu et al. 2013).

Biofertilizers are related commonly to plant growth promotion and responses to abiotic stresses, induced by a pool of bioactive compounds from a great diversity of environment friendly sources. The beneficial bacteria can produce phytohormones and other compounds (Borriss 2011), or biomasses and their extracts, (*e.g.*) algae (Jannin et al. 2013) and yeast (Lonhienne et al. 2014), or by mycorrhizal fungi (Bettoni et al. 2014), even products obtained by fermentation as an amino acid sources (Civiero et al. 2013), among a huge diversity of sources that nature and the biotechnology can offer.

Under the same concept, the biopesticides defined by the US Environmental Protection Agency (EPA) as pesticides derived from natural materials (Borriss 2011), that in general are no pathogenic microorganisms strains (Vinale 2014) or plant extracts (Kasiotis 2013) with effect against pests or diseases, or the bio-inoculants related to biologic nitrogen uptake, are called sometimes as biofertilizers too.

The biofertilizers definition on regulatory affairs not exactly specify the sources, but for example in Brazilian regulation determine the bioactivity as a main effect: "*Biofertilizer is a product that contains active ingredient or organic agent, free for agrochemicals, capable of act directly or indirectly on all or part of cultivated plants, raising the productivity, without taking into account their hormonal or stimulating value*" (Brasil 2004). On Brazilian regulation of organic production, Biofertilizer is defined as a *"product containing active components or biological agents capable to acting, directly or indirectly, on the whole or part of cultivated plants, improving the performance of the production system and that been free from substances prohibited by the rules of organic production"* (Brasil 2008). In both of regulations, the bioactivity and/or some active ingredients is needed to characterize a biofertilizer.

According Balachandar (2012) even though hundreds of bacteria and fungi were identified for enhancing plant growth, only few have been commercially exploited as biofertilizers. In the same way, many natural compounds could be classified as biofertilizer with proven bioactivity, such as: fulvic acid, amino acids and kelp extracts. These compounds are sold as common mixed fertilizer with mineral nutrients, and their bioactivity is not observed.

To stimulate researchers and companies for finding new biofertilizer sources, and deliver them according regulations to the market as a sustainable tool to the growers, the characterization of the plant growth promotion (PGP) and distinction from biopesticides, bio-inoculants, mineral fertilizers and biostimulants is desirable.

The establishment of simple bioassays to find and characterize the PGP effect before the field trials could be an efficient tool on biofertilizers research. The bioassays developed from 60s to 90s, following the development of plant hormones and plant growth regulators knowledge, could be very useful in screenings to find PGP bioactivity on potential biofertilizer sources, as the classical bioassay described by Zhao et al. (1992), which uses cucumber (*Cucumis sativus*) hypocotyl and cotyledons evaluating expansion after excision of whole seedlings used to find growth effect by action of the tested substances. In the same way, Stirk (2002) got results with cucumber cotyledon root formation using Cyanophyta and microalgae extracts, and Sharma et al. (2012) shows the bioactivity of brown seaweed species with bioassay of extracts using mung bean (*Vigna radiata*) and pak choi (*Brassica rapa chinensis*).

The clear characterization of biofertilizer related to their bioactivity, and the consolidation of nomenclature of biofertilizer in both scientific and regulatory literature as a class of natural source bioactive products, could consolidate this eco-friendly technology to the new agriculture. Focused in search and characterization of bacteria to potential use in

agriculture, showing PGP effect, or as biofertilizer, bio-inoculant or even biopesticide, some strategies are discussed forward.

2.1.2 Biofertilizer's Characteristic - Siderophores

Biofertilizers characteristics are known for their ability to provide the plant root with nutrients such as nitrogen, phosphorous and iron.

Iron is the fourth most abundant element on earth, in aerobic soils, iron is not readily assimilated by bacteria or plants. This element can exist in aqueous solution in two states: Fe^{2+} and Fe^{3+}. The Fe^{3+} forms are not utilizable by plants and microorganisms because they often form insoluble oxides or hydroxides limiting your bioavailability (Ma 2005; Zuo and Zhang 2011). The Iron is an essential nutrient for plants and its deficiency is exhibited in severe metabolic alterations, mainly because iron is present as a cofactor in many enzymes essential to physiological processes, such as respiration, photosynthesis and nitrogen fixation (Taiz and Zeiger 2009).

Microorganisms and plants requires a high level of iron, and to obtain sufficient iron is even more complicated in the rhizosphere, because at this site, plant, bacteria and fungi compete for nutrient, in this way the siderophores may act directly in the growth promotion and indirectly in biological control (Guerinot and Ying 1994; Hu and Xu 2011).

Plants can use two strategies to acquire iron: (i) acidification of the rhizosphere followed by reduction of Fe^{3+} ions by membrane-bound Fe(III)-chelate reductase and uptake of Fe^{2+} into root cells; (ii) plants secrete low molecular weight phytosiderophores for solubilization and bind iron which is then transported into root cells via membrane proteins (Altomare and Tringovska 2011). However, these strategies are often not efficient enough to the necessity of plants growing especially in calcareous or alkaline soils. Therefore, in this case it is necessary providing plants accessible forms of iron (Zuo and Zhang 2011).

Microorganisms also secrete siderophores due the low disponibility of Fe^{+3} in solution. The bacterial growth as well as siderophore production is stimulated by $(NH_4)_2SO_4$ (ammonium sulphate) and amino acids, however, the optimum siderophore yield is obtained with urea (Sayyed et al. 2005). Many siderophores may form complexes with some elements such as copper, aluminum and molybdenum. (Benite et al. 2002). These elements may act on the external side of cell membrane, binding iron molecules in solution with specifically membrane

receptor, where they are absorbed, thereby making iron available for growth promotion in plant (Taiz and Zeiger 2009).

There are over than 500 known siderophores and the chemical structures of 270 of these compounds have been determined (Hider and Kong 2010). Production of siderophores by bacteria is detected via the chrome azurol S assay, a general test, which is independent of siderophore structure. Siderophores are usually classified by the ligands used to chelate the ferric iron: catecholates (phenolates); hydroxamates; and carboxylates (*e.g.* derivatives of citric acid) (Taiz and Zeiger 2009).

Glick (2012) defined the benefits of bacterial siderophores to plants using examples of different experiments. The experiments cited including benefits in mung bean (*Vigna radiate*), peanut (*Arachis hypogaea*) and *Arabidopsis thaliana* plants. In mung bean plants the *Pseudomonas* produced the siderophore, growing in iron-limited condition, enhancing chlorophyll contents in plant and reducing chlorotic aspect in leaves (Sharma et al. 2003). Either the specie *Pseudomonas putida* also reduced chlorotic aspect in peanut, when iron deficiency was induced (Jurkevitch et al. 1992). Likewise, *Pseudomonas fluorescens* helped to better performance in *Arabidopsis thaliana*, raising iron contents in plant tissues, mediated by the bacterium Fe-pyoverdine complex inside plants (Vansuyt et al. 2007).

The provision of iron to plants by bacteria is even more important when the plants suffer an environmental stress (*e.g.* heavy metal pollution). In this situation, siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals (Braud et al. 2006; Ines et al. 2012).

2.1.3 Phytostimulator's Characteristic – Auxins

Plant hormones are a group of naturally occurring organic substances that influence physiological processes at low concentrations in response to the environment stimulus (Davies 2004). When this plants responses are not so effective, rhizosphere microorganisms may also produce or modulate phytohormones (Salamone et al. 2005) so that many bacterias can alter phytohormone levels and thereby affect the plant's hormonal balance and its response to environment (Glick et al. 2007).

The indole acetic acid (IAA) is the main auxin in natural occurence plants (Taiz and Zeiger 2009). IAA may act in many physiology process in the plants such as: affects photosynthesis and pigment formation; mediates responses to light, gravity and florescence;

controls biosynthesis of various metabolites; modules resistance to stressful conditions; controls processes of vegetative growth; more specifically act in cell division and differentiation, stimulates seed and tuber germination, increases the rate of xylem and root development, initiates lateral and adventitious root formation (Spaepen and Vanderleyden 2011; Taiz and Zeiger 2009). Howsoever, countless bacteria are still able to synthesize IAA, such as: *Azospirillum brasilienses*, *A. lipoferum* (Kuss et al. 2007) species of *Bacillus* and *Paenibacillus* (Beneduzi et al. 2008); *Providencia* (Rana et al. 2011) and *Pseudomonas fluorescens* (Hernández-Rodríguez et al. 2008). In general, there is a partnership between the plant and the bacteria, as in the situation where the bacterial IAA increases root surface area and length, providing the plant greater access to soil nutrients. In addition, bacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation, allowing more nutrients to support the growth of rhizosphere bacteria (Glick 2012).

The response of the plant to IAA varies with the type, degree of sensitivity, developmental stage of the plant and according to the particular tissue involved, for example, in roots versus shoots (the optimal level of IAA for supporting plant growth is ~5 orders of magnitude lower for roots than for shoots) (Taiz and Zeiger 2009). However, the endogenous pool of plant IAA may be altered by the acquisition of bacterial IAA. The level of IAA synthesized by the plant is important in determining whether bacterial IAA stimulates or suppresses plant growth (Glick 2012).

For example: canola (*Brassica campestris*) seeds inoculated with wild-type *Pseudomonas putida* increased the length of roots compared with an IAA-deficient mutant and the control uninoculated (Xie et al. 1996); when the same strain was inoculated in mung bean (*Vigna radiate*) cuttings with a mutant which overproduces IAA, yielded a much greater number of shorter roots compared with controls (Mayak et al. 1999). Or even with the use of purified bacterial auxins of *B. subtilis* and *B. licheniformis* also has an influence on plant growth of red-pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*), displaying up to 20% increased root, stem, and leaf growth (Lim and Kim 2009)**.**

Root nodulation is also affected by IAA, most rhizobia strains that have been examined have been found to produce IAA (Badenoch-Jones et al. 1984; Boot et al. 1999; Datta and Basu 2000) and several studies have suggested that increases in auxin levels in the host plant are necessary for nodule formation (Mathesius et al. 1998; Pii et al. 2007; Mathesius 2008). Soybean plants inoculated with *Bradyrhizobium* spp. mutant that had a decreased level of IAA synthesis had a lower nodule mass and fixed less nitrogen per gram of nodule (Hunter 1987),

and induced fewer nodules on soybean roots (Fukuhara et al. 1994) than did plants inoculated with wild-type bacteria, supporting the idea that part of the IAA found in nodules is of prokaryotic origin and that this IAA facilitates nodulation.

The bacterial IAA not only serves to manipulate host physiology but also acts as a bacterial signal (Spaepen et al. 2007). Interesting in this context is the stimulation by IAA of its own synthesis in *Azospirillum* species, analogous to a *quorum sensing* (QS) or auto activation mechanism. This hypothesis has calling attention for the plant-associated bacteria that can actively destroy IAA and can be quite common on plant (Riviere and Berthier 1964), such as *Alcaligenes*, *Pseudomonas* (Libbert and Risch 1969), *Arthrobacter* (Mino 1970), and *Bradyrhizobium* (Egebo et al. 1991) . Some, like *Pseudomonas putida*, can use IAA as a sole source of carbon, nitrogen, and energy (Laveau and Lindow 2005).

2.1.4 References

Altomare C, Tringovska I (2011) Beneficial soil microorganisms, an ecological alternative for soil fertility management. In: Lichtfouse E (ed) Genetics, biofuels and local farming systems. Sustainable agriculture reviews, vol 7. Springer, Netherlands, p 161-214

Badenoch-Jones J, Summons RE, Rolfe BG, Letham DS (1984) Phytohormones, Rhizobium mutants, and nodulation in legumes. IV. Auxin metabolites in pea root nodules. J Plant Growth Regul 3:23-39

Balachandar D (2012) Biofertilizers - What Next? J Biofertil Biopestici. doi:org/10.4172/2155-6202.1000e108

Beneduzi A, Peres D, Vargas LK, Bodanese-Zanettini MH, Passaglia LMP (2008) Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. Appl Soil Ecol 39:311-320

Benite AMC, Machado SP, Machado BC (2002) Sideróforos: Uma resposta dos microorganismos. Quim Nova 25:1155-1164

Bettoni MM, Mógor ÁF, Pauletti V, Goicoechea N (2014)Growth and metabolism of onion seedlings as affected by the application of humic substances, mycorrhizal inoculation and elevated CO_2. Sci Hort 180:227-235

Bevivino A, Dalmastri C, Tabacchioni S, Chiarini L (2000) Efficacy of *Burkholderia cepacia* MCI 7 in disease suppression and growth promotion of maize. Biol Fert Soils 31:225-231

Boot KJM, van Brussel AAN, Tak T, Spaink HP, Kijne JW (1999) Lipochitin oligosaccharides from *Rhizobium leguminosarum* bv. *viciae* reduce auxin transport capacity in *Vicia sativa* subsp *nigra* roots. Mol Plant Microbe Interact 12:839–844

Borriss R (2011) Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents in agriculture In: Maheshwari DK (ed.) Bacteria in agrobiology: plant growth responses, Springer, Berlin p 41-76

Brasil 2004. Ministério da Agricultura, Pecuária e Abastecimento. Lei de Fertilizantes, Corretivos, Inoculantes, Estimulantes ou Biofertilizantes. Decreto nº 4.954 de 14 de janeiro de 2004, Brasília

Brasil 2008. Ministério da Agricultura, Pecuária e Abastecimento. Regulamento Técnico que estabelece as normas técnicas para os Sistemas Orgânicos de Produção. Instrução Normativa nº 64 de 18 de dezembro de 2008, Brasília

Braud A, Jézéquel K, Vieille E, Tritter A, Lebeau T (2006) Changes in extractability of Cr and Pb in a polycontaminated soil after bioaugmentation with microbial producers of biosurfactants, organic acids and siderophores. Water Air Soil Pollut 6:261–279

Civiero JC et al. (2013) Stem and root growth of sugar cane for the use of humic acid and L-glutamic acid. Braz J Appl Technol Agric Sci, 6(1):47-51

Datta C, Basu PS (2000) Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. Microbiol Res 155(2):123-127

Davies PJ (2004) The plant hormones: their nature, occurrence, and functions. In: Davies PJ (ed) Plant hormones, biosynthesis, signal transduction, action. Kluwer Academic Publishers, Dordrecht, p 1-15

Egebo LA, Nielsen SVS, Jochimsen BU (1991). Oxygen-dependent catabolism of indole-3-acetic acid in *Bradyrhizobium japonicum*. J Bacteriol 173:4897–4901

Fukuhara H, Minakawa Y, Akao S, Minamisawa K (1994) The involvement of indole-3-acetic acid produced by *Bradyrhizobium elkanii* in nodule formation. Plant Cell Physiol 35(8):1261-1265

Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119(3):329-339

Glick BR (2012) Plant Growth-Promoting Bacteria: Mechanisms and Applications. Scientifica. doi:10.6064/2012/963401

Guerinot ML, Ying Y (1994) Iron: nutritious, noxious, and not readily available. Plant Physiol 104(3):815-820

Hernandez-Rodriguez A, Heydrich-Perez M, Acebo-Guerrero Y, Velazquez-Del Valle MG, Hernandez-Lauzardo AN (2008) Antagonistic activity of Cuban native rhizobacteria against *Fusarium verticillioides* (Sacc.) Nirenb. in maize (*Zea mays* L.). Appl Soil Ecol 39(2):180-186

Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27(5): 637-657

Hu QP, Xu JG (2011) A simple double-layered chrome azurol S agar (SD-CASA) plate assay to optimize the production of siderophores by a potential biocontrol agent *Bacillus*. Afr J Microbiol Res 5(25):4321-4327

Hunter WJ (1987) Influence of 5-methyltryptophan-resistant *Bradyrhizobium japonicum* on soybean root nodule indole-3-acetic acid content. Appl Environ Microbiol 53:1051-1055

Ines M et al. (2012) Effect of dose-response of zinc and manganese on siderophores production. Am J Environ Sci 8:143-151

Jannin L et al. (2013) *Brassica napus* growth is promoted by *Ascophyllum nodosum* (L.) Le Jol. seaweed extract: microarray analysis and physiological characterization of N, C, and S metabolisms. J Plant Growth Regul 32(1):31-52

Jurkevitch E, Hadar Y, Chen Y (1992) Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria. Appl Environ Microbiol 58(1):119-124

Kasiotis KM (2013) Biopesticides Analysis: An Editorial. J Biofertil Biopestici. doi:org/10.4172/2155-6202.1000e115

Kuss AV, Kuss VV, Lovato T, Flôres Ml (2007) Fixação de nitrogênio e produção de ácido indol acético in vitro por bactérias diazotróficas endofíticas. PAB 42:1459-1465

Leveau JHJ, Lindow SE (2005) Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. Appl Environ Microbiol 71:2365-2371

Libbert E, Risch H (1969) Interactions between plants and epiphytic bacteria regarding their auxin metabolism. V. Isolation and identification of the IAA-producing and -destroying bacteria from pea plants. Physiol Plantarum 22:51-58

Lim JH, Kim SD (2009) Synergistic plant growth promotion by the indigenous auxins-producing PGPR *Bacillus subtilis* AH18 and *Bacillus licheniforims* K11. J Korean Soc Appl Bi 52(5):531-538

Lonhienne T et al. (2014) Yeast as a biofertilizer alters plant growth and morphology. Crop Science 54(2):785-790

Ma JF (2005) Plant root responses to three abundant soil minerals: silicon, aluminum and iron. Crit Rev Plant Sci 24(4):267-281

Mathesius U et al. (1998) Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides.Plant J 14(1):23-34

Mathesius U (2008) Auxin: at the root of nodule development? Funct Plant Biol 35:651-668

Mayak S, Tirosh T, Glick BR (1999) Effect of wild-type and mutant plant growth-promoting rhizobacteria on the rooting of mung bean cuttings. J Plant Growth Regul 18(2):49-53

Mino Y (1970) Studies on the destruction of indole-3-acetic acid by a species of *Arthrobacter*. IV. Decomposition products. Plant Cell Physiol 11:129-138

Rana A et al. (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol 61:893-900

Riviere J, Berthier B (1964) Action des microorganismes de la rhizosphère sur la croissance du blé. III. Isolement et identification des bacteries dégradant l'acide indole-3-acétique. Ann Inst Pasteur 3:250-256

Salamone IEG, Hynes RK, Nelson LM (2005) Role of cytokinins in plant growth promotion by rhizosphere bacteria. In: Siddiqui ZA (ed) PGPR: Biocontrol and Biofertilization. Springer, Amsterdam, p 173-195

Sayyed RZ, Badguzar MD, Sonawane HM, Mhaske MM, Chincholkar SB (2005) Production of microbial iron chelators (siderophores) by fluorescent Pseudomonads. Indian J Biotechnol 4:484-490

Sharma A, Johri BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35(7):887-894

Sharma SHS et al. (2012) Biostimulant activity of brown seaweed species from Strangford Lough: compositional analyses of polysaccharides and bioassay of extracts using mung bean (*Vigno mungo* L.) and pak choi (*Brassica rapa chinensis* L.) J Appl Phycol 24:1081-1091

Simiyu NSW, Tarus D, Watiti J, Nang'ayo F (2013) Effective Regulation of bio-fertilizers and bio-pesticides: A potential avenue to increase agricultural productivity. International Institute of Tropical Agriculture, Compro II policy series n1

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425-448

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol. doi: 10.1101/cshperspect.a001438

Stirk WA, Ördög V, van Staden J, Jäger K (2002) Cytokinin- and auxin-like activity in Cyanophyta and microalga. J Appl Phycol 14:215-221

Taiz L, Zeiger E (2009) Fisiologia vegetal. 4rd edn, Artmed, Porto Alegre

Vansuyt G, Robin A, Briat JF, Curie C, Lemanceau P (2007) Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana.* Mol Plant Microbe In 20(4):441-447

Vinale F (2014) Biopesticides and biofertilizers based on fungal secondary metabolites. J Biofertil Biopestici. doi:org/10.4172/2155-6202.1000e119

Xie H, Pasternak JJ, Glick BR (1996) Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. Curr Microbiol 32(2):67-71

Zhao ZR, Wu ZL, Huang GQ, Li GR (1992) An improved disk bioassay for determining activities of plant growth regulators. J Plant Growth Regul 11:209-213

Zuo Y, Zhang F (2011) Soil and crop management strategies to prevent iron deficiency in crops. Plant Soil 339:83-95

2.2 Plant growth promoting bacteria and tomato interaction in different development stages

Full text on: SZILAGYI-ZECCHIN, V. J.; MÓGOR, Á. F. How bacteria, as an eco-friendly tool, can contribute to the sustainable tomato cultivation?. In: KUMAR, V.; KUMAR M.; SHARMA, S. (eds) Probiotics in Agroecosystem. Springer, 2017.

2.2.1 Seed germination

The first stage of plant development is the seed germination, where many physiological processes starting to a new plant arise. In this stage, the bacteria can act on germination and plantlets growth and some studies with different bacterial genus that will be seen below can show it.

Pseudomonas oleovorans strains, tested on paper towel germination assay, improved the tomato seeds germination, cvs Arka Vikas and Arka Abha, while *Agrobacterium tumefaciens* strains displayed higher seedling fresh weight, improving the tomato seedlings vigor index in the same cultivars (Thomas and Upreti 2015). Also *Pseudomonas aeruginosa* and *Bacillus subtilis* showed improvement in tomato germination rate (Adesemoye et al. 2008).

In the same way, *Rhodopseudomonas* sp. improved the germination percentage of seed, total length, and dry mass of germinated tomato seedling were increased by 30.2%, 71.1%, and 270.8%, respectively, compared with those of the uninoculated control at 7 days after inoculation. This purple nonsulfur bacteria, produced respectively 5.56 mM/min/mg of protein and 67.2 μM/min/mg of protein both IAA and 5-aminolevulinic acid (ALA), in which may be one of the mechanisms for tomato plants growth enhancement (Koh and Song 2007).

The strains *Bacillus subtilis* GBO3, *Bacillus amyloliquefaciens* IN937a and *Brevibacillus brevis* inoculated on tomato seeds, showed enhancement in the seed quality parameters like seed germination and seedling vigor (Girish and Umesha 2005).

Tomato seeds cv. Liso Marglove inoculated with *Azospirillum brasilense* FT326, analysed 15 days after inoculation, identify that FT362 were localized on roots and within xylematic tissue, promoting increases in shoot and root fresh weight, and root hair length. Also the levels of indole-3-acetic acid (IAA) and ethylene were higher in inoculated plants (Ribaudo et al. 2006).

2.2.2 Seedlings production

The tomato seedling production, period in general around 30 to 40 days after germination, is well studied because it commercial importance. Accelerate the seedling growth in the nursery contributes to healthier and vigorous seedlings, which in turn to facilitates better establishment of plants on the field, that can reflect on better yield at the end of cicle (Thomas and Upreti 2015). Therefore, effects of bacteria in this stage are intended and can be obtained, because the bacteria can change many aspects of primary and secondary metabolism of the plant (Szilagyi-Zecchin et al. 2016).

In this regard, the effect of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, in two concentrations were evaluated in the production of organic seedlings of two tomato cultivars through seeds inoculation. This strain, was positive for production of indole compounds and siderophores, enhanced the contents of chlorophyll in the seedling leaves and promoted shoot growth with additions of 47.7% for ‘Santa Clara’ and 15.5% for ‘Cereja’ when compared to the control (Szilagyi-Zecchin et al. 2015a).

The bacteria *Sphingomonas* sp. LK11, release physiologically active gibberellins GA4 and inactive GA9 and GA20, and also produce IAA. When inoculated on tomato seeds showed significantly increases on shoot length, chlorophyll contents, shoot, and root dry weights on 5 weeks old plants (Khan et al. 2014).

In addition, some endophytic bacteria *Bacillus* sp. BETL9, *Serratia marcescens* BECL8, both phosphate solubilizer and *Bacillus pumilus* BETL13, *Bacillus licheniformis* BECS1, and *Bacillus megaterium* BECS7, all siderophores and IAA releasers, were tested on tomato, in which, three weeks after the emergence were observed increases in root and shoot length and in the number of secondary roots (Amaresan et al. 2012).

2.2.3 Intermediate development stage

Researches focusing the development after seedling stage are elucidated in part how bacteria strains acts near to the flowering or in the begining of fruiting, but they are so scarce.

Adesemoye et al. (2008) comparing *Pseudomonas aeruginosa* and *Bacillus subtilis* on seeds inoculation, were found that the dry biomass of the tomato plants at 65 days after sowing was increased around 31% by both bacteria.

Tomato plants cv. Río Fuego cultivated in greenhouse and inoculated with *Bacillus subtilis* BEB-lSbs had the radicular system 50 days after transplant, improved in root dry weight and root length by 26% and 15%, respectively (Mena-Violante and Olalde-Portugal 2007).

2.2.4 Yield

The main aim of the use of bacteria is improve the yield by inoculation, and sometimes reducing the use of synthetic fertilizers. As was reported above, it is possible, and also it is feasible by the following studies.

The gram positive bacteria strains, specially using *Bacillus* genus, could be used to improve nutrients uptake because they have some abilities like endospore formation that can provide more viability to formulate and to survive in inhospitable environments, as sandy saline soils.

Adesemoye et al. (2009), using 75% of the recommended fertilizer rate in association with *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4 inoculation, had tomato (cultivar Juliet) yield and nutrient (nitrogen and phosphorus) uptake equivalent to the full fertilizer rate without inoculation.

Also with application of other *B. amyloliquefaciens* FZB24 and FZB42 strains, Gül et al. (2008) obteined increased the yield of the tomato plants (Durinta cultivar) by 8-9%.

The cultivar Río Fuego had higher yield per plant (around 23% more) and marketable yield when inoculated with *Bacillus subtilis* BEB-lSbs (Mena-Violante and Olalde-Portugal 2007).

García et al. (2004), inoculating *Bacillus licheniformis* CECT 5106 applied on two tomato varieties (‘Daniela’ and ‘Brillante’), found that the bacteria increased the height and the leaf area in both cultivars. Also in greenhouse assay, those authors found that the number and diameter of tomato fruits produced in sand and in hydroponic medium were increased significantly by inoculation with CECT 5106.

Not only gram positive bacteria has agronomic abilities to improve the tomato yield, some genus gram negative has also been reported.

Pseudomonas putida B strain 1, IAA producer, was evaluated to determine the promoting effect on the growth of mature healthy tomato plants, cv. Trust F1, under hydroponic conditions. It shown to improve fruit yields in rockwool (688 g per plant) and in organic medium (630 g per plant) (Gravel et al. 2007).

Siderophores from *Chryseobacterium* sp. C138 isolated from the rhizosphere of rice (*Oryza sativa*) are effective in supplying Fe to iron deficient tomato plants of var. Marglobe by the roots on experiment conducted in greenhouse under iron hydroponic conditions. The media free of bacteria and with bacterial cells were applied and both significantly increased plant yield, chlorophyll and iron content over the positive controls with full Hoagland solution (Radzik et al. 2013).

The *Burkholderia tropica* strain MTo-293, isolated from maize stems, able to colonize the root hairs, root tips, lateral root emergence sites, and stomata of tomato leafs, was used in two tomato crop season in a greenhouse experiments, and showed a consistent increase of both number and weight of fruits. The number of fruits, considering the two seasons was increased at average of five fruits per m^2, and regarding to a higher value range fruits, the increase were of 7.6 fruits per m^2 in the second season (Bernabeu et al. 2015).

2.2.5 References

Adesemoye AO, Obini M, Ugoji EO (2008) Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. Braz J Microbiol 39(3): 423-426

Adesemoye AO, Torbert H., Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb ecol 58(4): 921-929

Amaresan N, Jayakumar V, Kumar K, Thajuddin N (2012) Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. Ann Microbiol 62(2):805-810

Bernabeu PR, Pistorio M, Torres-Tejerizo G et al (2015) Colonization and plant growth-promotion of tomato by *Burkholderia tropica*. Sci Hort 191:113-120

García, JAL, Probanza A, Ramos B, Palomino M, Maņero FJG (2004) Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. Agronomie 24(4):169-176

Girish N, Umesha S (2005) Effect of plant growth promoting rhizobacteria on bacterial canker of tomato. Arch Phytopathol Plant Protect 38(3):235-243

Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39(8):1968-1977

Gül A, Kidoglu F, Tüzel Y (2008) Effects of nutrition and *Bacillus amyloliquefaciens* on tomato (*Solanum lycopersicum*, L.) growing in perlite. Span J Agric Res 6(3):422-429

Khan AL, Waqas M, Kang SM et al (2014) Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. J Microbiol 52(8):689-695

Koh RH, Song HG (2007) Effects of application of *Rhodopseudomonas* sp. on seed germination and growth of tomato under axenic conditions. J Microbiol Biotechnol 17(11):1805-1810

Mena-Violante HG, Olalde-Portugal V (2007) Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. Sci Hort 113(1):103-106

Radzki W, Manero FG, Algar E et al (2013) Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. A Van Leeuwenhoek 104(3): 321-330

Ribaudo CM, Krumpholz EM, Cassán FD et al (2006) *Azospirillum* sp. promotes root hair development in tomato plants through a mechanism that involves ethylene. J Plant Growth Regul 25(2):175-185

Szilagyi-Zecchin VJ, Mógor ÁF, Ruaro L, Röder C (2015a) Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 em cultura orgânica. Rev Ciênc Agrar 38(1):26-33

Szilagyi-Zecchin VJ, Mógor ÁF, Figueiredo GGO (2016) Strategies for characterization of agriculturally important bacteria. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity. Springer India, p 1-21

Thomas P, Upreti R (2015) Evaluation of tomato seedling root-associated bacterial endophytes towards organic seedling production. Organic Agri 1-10

3 CAPÍTULO I – CRESCIMENTO DE MUDAS DE TOMATEIRO (*Solanum lycopersicum*) ESTIMULADO PELA BACTÉRIA *Bacillus amyloliquefaciens* SUBSP. *plantarum* FZB42 EM CULTURA ORGÂNICA

SCAP
SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 em cultura orgânica

Tomato seedlings growth (*Solanum lycopersicum*) promoted by bacteria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 in organic system

Vivian J. Szilagyi-Zecchin*, Átila Francisco Mógor, Lucimeris Ruaro e Cinthia Röder

Departamento de Fitotecnia e Fitossanitarismo, Universidade Federal do Paraná, Rua dos Funcionários, 1540 – Caixa Postal 19061, CEP 80035-050, Curitiba, Paraná, Brasil, E-mail: vivian.szilagyi@gmail.com, author for correspondence

RESUMO

Este estudo verificou a atuação da bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, na produção de mudas orgânicas de duas cultivares ('Santa Clara I-5300' e 'Cereja 261') de tomateiro (*Solanum lycopersicum* L.) mediante a inoculação nas sementes. Também se investigaram as características bacterianas relacionadas com a promoção do crescimento vegetal. Para isso, utilizou-se uma solução de FZB42 na concentração de $1x10^{11}$ UFC/mL, com os tratamentos correspondendo a porcentagens em volume (20% de FZB42; 80% de FZB42 e Testemunha com 100% de água destilada), na proporção de 320 uL/g de sementes. O percentual de germinação não foi influenciado pelos tratamentos e verificou-se estímulo ao crescimento da parte aérea por FZB42 a 20% nas plântulas da cv 'Cereja'. Na produção de mudas, a bactéria FZB42 a 20% promoveu o crescimento enquanto a FZB42 a 80% o reduziu. A inoculação nas duas doses aumentou os teores clorofila *a*, *b* e totais das mudas de tomateiro. A estirpe FZB42 apresentou resultados positivos para produção de compostos indólicos e sideróforos, e na dose de 20%, mostrou-se vantajosa na produção de mudas, ao aumentar a parte aérea em 47,7% na cultivar 'Santa Clara', e 15,5% na cv 'Cereja', quando comparados à testemunha.

Palavras-chave: sistema orgânico, bactérias promotoras do crescimento vegetal, inoculante, *Solanum lycopersicum*.

ABSTRACT

This study we sought to evaluate the action of the bacteria *Bacillus amyloliquefaciens* subsp. *plantarum* in the production of organic seedlings of two cultivars ('Santa Clara I-5300' and 'Cereja 261') from tomato (*Solanum lycopersicum* L.) through inoculated seeds. And investigate bacterial characteristics related to plant growth promotion. For this, we used FZB42 solution at a concentration of $1x10^{11}$ CFU/mL, with treatments correspond to percentages by volume (20% FZB42; 80% of FZB42 and control 100% distilled water) at a ratio of 320 uL/g seed. The germination percentage was not influenced by the treatments, and FZB42 to 20% increased the shoot in cultivar 'Cereja'. In seedling production, FZB42 to 20% promoted growth while FZB42 to 80% decreased it. Inoculation with the two doses tested of FZB42 enhanced the contents of chlorophyll *a*, *b* and total in the tomato seedling. The FZB42 strain showed positive results for production of indole compounds and siderophore; and in a dose of 20% was more advantageous to the tomato seedling, promoting shoot growth with added of 47.7% for 'Santa Clara' and 15.5% for 'Cereja' when compared to the control.

Keywords: organic system, plant growth promoting bacteria, inoculant, *Solanum lycopersicum*.

3.1 Introdução

O tomateiro (*Solanum lycopersicum L.*) é cultivado no mundo inteiro e possui grande relevância econômica e social (FAOSTAT, 2010). É a hortaliça mais industrializada, nas formas de suco, molho, pasta, e desidratada (FAOSTAT, 2011). Além de ser um alimento funcional devido aos altos teores de vitaminas A, C e licopeno (Carvalho e Pagliuca, 2007).

A qualidade dos alimentos (frescos ou processados) vem sendo considerada fator de segurança alimentar e nutricional, relacionada não só a produção em quantidades suficientes e acesso garantido, mas também à promoção do estado de saúde daqueles que os consomem (Souza e Resende, 2006), impulsionando o mercado de produtos orgânicos, com destaque para as hortaliças, como o tomate (Toledo *et al*., 2011).

No cultivo do tomateiro, a produção de mudas é uma das etapas mais importantes (Silveira *et al*., 2002). De acordo com Minami (1995), 60% do sucesso de um cultivo depende do plantio de mudas de boa qualidade. Assim, ganham importância as técnicas que promovam o crescimento adequado das mudas, especialmente no sistema orgânico, dependente de insumos alternativos, como o uso de microrganismos benéficos.

Na literatura, são descritas algumas bactérias que vivem associadas às plantas e têm a habilidade de promover o crescimento vegetal (Compant *et al*., 2010). Espécies do gênero *Bacillus* vêm sendo utilizados para este fim: Mena-Violante e Olalde-Portugal (2007) verificaram efeitos positivos em frutos de tomate, como tamanho e a textura com aplicação de *B. subtilis*; Silva *et al*. (2008) usando *B. pumilus* observaram incrementos na altura das plantas de tomateiro nos estágios iniciais do desenvolvimento; e com uso de *Bacillus megaterium* (Harthmann *et al*., 2010) e *Bacillus cereus* (Harthmann *et al*., 2009) foi possível aumentar o rendimento de bulbos de cebola.

A estirpe FZB42 de *Bacillus amyloliquefaciens* subsp. *plantarum* vem sendo intensamente estudada, teve seu genoma sequenciado e por sua constituição, revelou um potencial para produzir metabolitos secundários, sendo mais de 8,5% do genoma dedicado a síntese de antibióticos e sideróforos (Chen *et al*., 2007). Seu filtrado promoveu o crescimento de coleóptilos de milho, devido à produção de bioativos como o ácido-indol-acético (Idris *et al*., 2004), e a aplicação de solução de esporos desta estirpe em sementes de tomate proporcionou acréscimos no rendimento de frutos em torno de 8 a 9% (Gül *et al*., 2008).

No presente trabalho, inoculou-se a bactéria *B. amyloliquefaciens* subsp. *plantarum* FZB42 em sementes de duas cultivares de tomateiro (Santa Clara e Cereja), com o objetivo de

verificar seu efeito na germinação e na produção de mudas em sistema orgânico, além de investigar características bacterianas relacionadas à promoção do crescimento vegetal.

3.2 Material e Métodos

Utilizou-se a bactéria *B. amyloliquefaciens* subsp. *plantarum* FZB42 (Omex® Agrifluids do Brasil Ltda), isolado de solo (Krebs *et al*., 1998) e depositado como estirpe 10A6 na coleção de culturas *Bacillus* Genetic Stock Center (BGSC, Ohio, E.U.A.).

As cultivares de tomateiro de crescimento indeterminado empregadas foram: Cereja 261 (Isla®), ciclo de 90 dias; e Santa Clara I-5300 (Isla®) com ciclo de 110 dias. A empresa fornecedora de sementes (sem tratamento químico) garantiu 84% de germinação e 100% de pureza para ambas as cultivares.

3.2.1 Testes bioquímicos na bactéria

A quantificação de compostos indólicos foi determinada em meio líquido enriquecido com triptofano e utilizando o reagente de Salkowski segundo Szilagyi-Zecchin *et al.* (2014). A concentração dos compostos foi estimada por curva-padrão com quantidades conhecidas de acido indol acético (Sigma-Aldrich®) que variou entre 0 e 30 µg/mL de acordo com a equação $y = 0{,}0057\ x$ ($R^2 = 1$). E como controle positivo utilizou-se *Azospirillum brasilense* Ab-V5.

Para produção de sideróforos, usou-se o método colorimétrico universal de Schwyn e Neilands (1987) de acordo com as adaptações de Szilagyi-Zecchin *et al.* (2014).

3.2.2 Teste de germinação

As sementes foram inoculadas com a solução de FZB42 na concentração de $1x10^{11}$ UFC/mL, sem prévia assepsia. Em seguida deixadas para secar à sombra sobre folhas de papel toalha.

Logo após, as sementes foram acomodadas em caixas plásticas transparentes (tipo Gerbox) sobre duas folhas de papel (mata-borrão) umedecidos com quantidade de água equivalente a 2,5 vezes o peso do papel seco (Brasil, 2009). E mantidas em B.O.D. a 25 °C, sem fotoperíodo. A avaliação contabilizando o número de plântulas normais e de plantas germinadas ocorreu aos 10 dias após semeadura.

O delineamento foi inteiramente casualizado em esquema fatorial 3 x 2 (dose de bactérias x cultivares de tomate). Possuindo 4 repetições de 100 sementes para cada cultivar, onde cada caixa era uma sub-repetição com 50 sementes.

Os tratamentos foram compostos das porcentagens da solução de FZB42, em volume de soluções aplicadas às sementes, sendo: (Testemunha) 100% de água destilada; (T1) 20% de FZB42 e 80% de água destilada; (T2) 80% de FZB42 e 20% de água destilada. As soluções corresponderam ao volume de 320 uL/g de sementes. A estimativa teórica de bactérias por semente para T1 e T2 foi de $1,6x10^5$ e $6,4x10^6$ respectivamente.

Após 10 dias avaliou-se o número de sementes germinadas e de plântulas normais. Nas plântulas consideradas normais a parte aera foi separada da raiz e foi mensurado: o comprimento (cm) e volume (cm^3) de raiz e parte aérea, por meio do software Win-rhizo® v. 4.0, acoplado a um Scanner LA1600 (Regent Systems, Quebec, Canadá) na resolução 150 dpi (Dots Per Inch ou Pontos Por Polegada).

3.2.3. Produção de mudas

A semeadura foi realizada em bandejas de poliestireno expandido, com 200 células. Estas foram preenchidas com, substrato comercial recomendado para hortaliças, preparado com composto de cama de aviário (Provaso®) e casca de pinus compostada (Vida Verde®) na proporção de 0,5:3,5 p/p.

As sementes foram inoculadas como descrito acima no teste de germinação e imediatamente semeadas. Distribuiu-se duas sementes por célula na profundidade de 1 cm. Após a germinação procedeu-se o desbaste. As mudas foram mantidas em casa vegetação com irrigação temporizada, na Área experimental de Olericultura Orgânica da Universidade Federal do Paraná (UFPR), no município de Pinhais-PR, durante o mês abril de 2013.

Em delineamento inteiramente casualizado, cada tratamento foi composto de 7 repetições com 40 células cada, e uma planta por célula.

Foram coletadas aos 33 dias após a semeadura, oito plantas centrais por repetição para a avaliação das seguintes variáveis biométricas: volume radicular (VR) expresso em cm^3; comprimento total de raízes (CTR) em cm, resultante da somatória dos comprimentos individuais das raízes; massa seca de raiz (MSR) expressa em g; área da parte aérea (APA) em cm^2 e massa seca da parte aérea (MSPA) em g. Para a obtenção do VR, CTR e APA as amostras foram analisadas por meio do programa computacional WinRhizo®. As folhas foram lidas na

resolução de 50 dpi e as raízes a 150 dpi. Para massa seca (raiz e parte aérea) estes foram levados à estufa com circulação de ar forçada, à temperatura de 65 ºC por 72 horas e em seguida pesados em balança de precisão analítica.

3.2.4 Determinação de clorofilas nas mudas

A determinação dos teores de clorofila foi realizada segundo Lichtenthaler (1987), utilizando todas as folhas das mudas (exceto as cotiledonares), de seis plantas por repetição. A absorbância foi lida em espectrofotômetro a 663 e 647 nm. Posteriormente, os valores foram transformados e expressos em mg de clorofila por g de material vegetal.

Os dados foram testados quanto à sua normalidade pelo teste de Kolmogorov-Smirnov e quanto à homogeneidade de variâncias, por Bartlett. Em seguida submetidos à análise de variância, e as médias foram comparadas pelo teste de Tukey, a 5% de significância no programa Assistat® 7.6 Beta (Silva e Azevedo, 2002).

3.3 Resultados e Discussão

A estirpe de *B. amyloliquefaciens* subsp. *plantarum* FZB42 produziu nas condições testadas 7,79 µg/mL de compostos indólicos enquanto que o controle *A. brasilense* produziu 31,42 µg/mL. FZB42 já foi descrita como produtora de compostos indólicos tais como, ácido indol acético e indol-3-acetonitrila, em outras condições de cultivo e por meio de diferentes métodos (Idris *et al*., 2004).

No presente trabalho, FZB42 secretou sideróforos, *in vitro,* em meio sólido. Já era conhecido que *B. amyloliquefaciens* subsp. *plantarum* FZB42 possuia potencial para produzir metabolitos secundários, pois mais de 8,5% do genoma é dedicado a síntese de antibióticos e sideróforos (Chen *et al*., 2007). Os sideróforos tem importância para as bactérias por suprirem a necessidade de ferro do próprio microrganismo, e para a sobrevivência no ambiente competitivo do solo (Khan *et al*., 2006).

Os tratamentos não alteraram significativamente o percentual de germinação informado no rótulo, pele fornecedor das sementes das duas cultivares. A quantidade de plântulas anormais, não diferiu entre os tratamentos, nem entre cultivares (dados não mostrados). A inoculação com FZB42 a 20% promoveu o aumento do volume da parte aérea das plântulas,

exclusivamente da cv Cereja, enquanto a dose de 80% reduziu o volume e comprimento da radícula e da parte aérea das duas cultivares (Figura 1).

Schlindwein *et al*. (2008), trabalhando com diferentes espécies de rizóbios em alface, verificaram que o percentual de germinação e a quantidade de plântulas normais também não foram influenciadas, exceto por *Rhizobium leguminosarum* biovar *trifolii*, que levou ao desenvolvimento de plântulas anormais com taxa de crescimento reduzido. No entanto, pode-se verificar influencias negativas sobre a germinação, como detectado por Miché *et al*. (2000) estudando duas estirpes de *A. brasilense*, estas impediram a germinação de sementes de striga (*Striga hermonthica)*, uma planta daninha, parasita obrigatório de cereais tropicais.

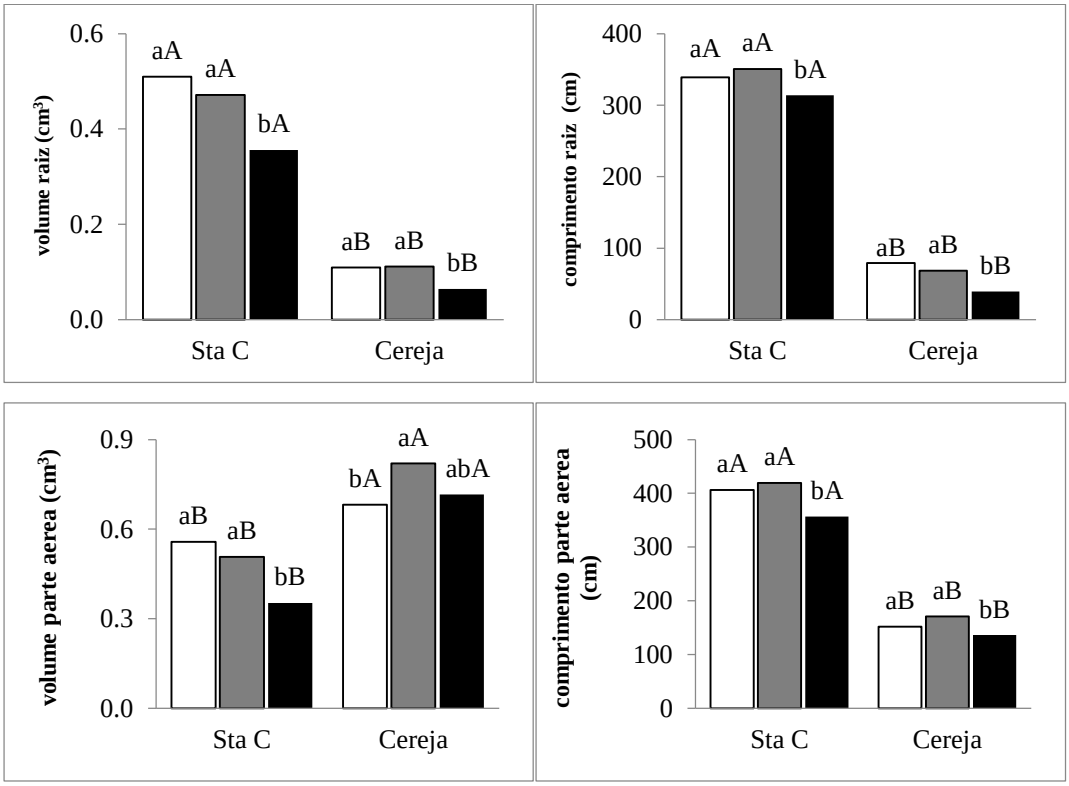

Figura 1. Avaliação de plântulas de tomateiro Santa Clara (Sta C) e Cereja, 10 dias após semeadura, em função das doses de FZB42 aplicadas à semente. Barras em branco = (Testemunha) 100% de água destilada; em cinza = (T1) 20% de FZB42; e em preto (T2) 80% de FZB42. Letras iguais, minúsculas entre os tratamentos e maiúsculas entre as cultivares, não diferem entre si pelo teste de Tukey a 1% de probabilidade.

Em contrapartida, existem trabalhos que relatam na germinação, estímulos positivos proporcionados por bactérias ao serem inoculadas nas sementes: Cassán *et al*. (2009) detectaram aumentos no percentual de germinação e no crescimento das plântulas de soja e

milho quando inoculadas com *A. brasilense* e *Brayrhizobium japonicum* sozinhos ou combinados; e Szilagyi-Zecchin *et al*. (2014) trabalhando com *Bacillus* sp. e *Enterobacter* sp. em milho verificaram aumento na germinação e no volume radicular das plântulas.

Na produção de mudas houve incrementos na área da parte aérea das duas cultivares com uso de FZB42 a 20%. Na cultivar Cereja FZB42 a 80%, houve redução da área em relação à testemunha (Quadro 1). A massa seca da parte aérea foi significativamente incrementada no tomate 'Santa Clara' com FZB42 a 20%, já FZB42 a 80% influenciou diminuindo a massa seca das duas cultivares.

Uma parte aérea maior permite uma melhor taxa fotossintética, que implica em mais fotoassimilados translocados para os órgãos em crescimento ou de reserva nos estádios seguintes (Taiz e Zeiger, 2004). Este aumento da parte aérea pode estar relacionado com a habilidade da bacteria estudada em produzir compostos indólicos. Alguns estudos demonstraram que *Bacillus* produtores de auxina trazem benefícios para a parte aérea do tomateiro, aumentando a altura, calibre e peso fresco de parte aérea de mudas (Domenech *et al*., 2006). Até mesmo quando a auxina bacteriana foi isolada, purificada e aplicada em mudas de tomate incrementaram em mais de 20% a parte aérea (Lim e Kim, 2009).

A inoculação de FZB42 testada contemplou duas quantidades de bactérias (T1 = $1{,}6x10^5$ e T2 = $6{,}4x10^6$ UFC/semente), o aumento da parte aérea ocorreu em T1, já em T2 um efeito oposto ocorreu nas mesmas variáveis, reafirmando um possível excesso de dose, com base no teste de germinação descrito acima.

Quadro 1. Desenvolvimento da parte aérea de mudas de tomateiro das cultivares 'Santa Clara'e 'Cereja' aos 33 dias após plantação de acordo com as doses de FZB42 aplicadas à semente. Test (100% de água destilada); T1 (FZB42 20%); T2 (FZB42 80%); (M) Média; (MG) Média Geral; (CV) Coeficiente de variação percentual.

A	*Raiz*								
	volume (cm3)			comprimento (cm)			massa seca (g)		
	Sta C	Cereja	Média	Sta C	Cereja	Média	Sta C	Cereja	Média
Test	4,27	3,21	3,74 a	1785,92	843,06	1314,49 a	0,14	0,15	0,15 a
T1	4,10	2,65	3,37 ab	1725,52	892,54	1309,03 a	0,14	0,13	0,13 a
T2	3,69	2,48	3,08 b	1624,99	769,44	1197,21 a	0,10	0,09	0,10 b
M	4,02 A	2,78 B		1712,14 A	835,01 B		0,13 A	0,12 A	
CV			13			10,69			15,5

Médias seguidas da mesma letra, maiúscula nas linhas e minúscula nas colunas, não diferem entre si pelo teste de Tukey a 1% de probabilidade.

Nas raízes das mudas, o volume e a massa seca foram inferiores a testemunha, quando inoculado com FZB42 a 80%. Enquanto FZB42 a 20% não diferiu da testemunha, para as três variáveis radiculares avaliadas (Quadro 2).

Essas diferenças podem estar relacionadas a uma entrada adicional de compostos indólicos produzidos pela bactéria, que modifica as quantidades endógenas na planta, para um nível ótimo ou acima do ótimo, resultando na indução ou inibição do crescimento vegetal (Patten e Glick, 1996). As raízes necessitam de uma concentração menor de compostos indólicos para crescer, e seu crescimento, pode ser inibido por concentrações que promovam o alongamento de caules e coleóptilos (Taiz e Zeiger, 2004).

Quadro 2. Desempenho do crescimento radicular de mudas de cultivares de tomateiro ‘Santa Clara’ (Sta C) e ‘Cereja’ aos 33 dias após com diferentes doses de FZB42 aplicadas à semente. Test (100% de água destilada); T1 (FZB42 20%); T2 (FZB42 80%); (M) Média; (MG) Média Geral; (CV) Coeficiente de variação percentual.

B	*Parte aérea*											
	área (cm2)						massa seca (g)					
	Sta C		Cereja		Média		Sta C		Cereja		Média	
Test	2812,12	cB	3858,40	bA	3335,26	b	0,71	bA	0,51	aB	0,61	b
T1	4154,64	aB	4458,08	aA	4306,36	a	0,93	aA	0,52	aB	0,72	a
T2	3411,22	bA	3121,87	cB	3266,55	b	0,65	cA	0,35	bB	0,50	c
M	3459,33	B	3812,78	aA			0,76	A	0,46	B		
CV					7,00						7,79	

Médias seguidas da mesma letra, maiúscula nas linhas e minúscula nas colunas, não diferem entre si pelo teste de Tukey a 1% de probabilidade.

Os teores de Cl *a*, *b* e totais foram superiores estatisticamente nas duas doses de bactéria em ‘Santa Clara’, enquanto para ‘Cereja’, somente FZB42 a 80% aumentou os níveis de clorofilas significativamente (Figura 2). Em média, nas duas cultivares há uma relação crescente entre os teores de clorofila e a quantidade de bactéria aplicada. As clorofilas estavam presentes em maiores teores nos tratamentos inoculados, mas com aumentos mais expressivos para a aplicação de FZB42 a 80%. Indicando que houve mais estimulo para produção de clorofila conforme o aumento da dose da bactéria, dentro dos limites testados.

Este padrão de resposta pode estar relacionado à produção de sideróforos pela bacteria. Pois, a planta aproveita estes compostos como quelantes orgânicos, que acabam a beneficiando, por disponibilizar o ferro para ser prontamente absorvido (Powell *et al*., 1980). Alguns exemplos deste mesmo mecanismo já foram observados em outras culturas. Uma delas, feijão

mungo (*Vigna radiata* L.) inoculado com *Pseudomonas* sp., produtora de sideróforos, e submetido a diferentes doses de ferro. Como resultado, teve suas quantidades de clorofilas *a*, *b* e totais aumentadas em 34, 48 e 39%, pois a bactéria melhorou a disponibilidade de ferro para a planta (Sharma *et al*., 2003). Similarmente, *Pseudomonas putida* reduziu a clorose induzida por deficiência de ferro em amendoim (*Arachis hypogaea* L.) (Jurkevitch *et al*., 1988).

Em todas as variáveis biométricas da produção de mudas, as respostas foram numericamente superiores no T1 (FZB42 20%) se comparadas ao T2 (FZB42 80%), e na maior parte delas (massa seca de raiz, área e massa seca da parte aérea), foram diferentes estatisticamente. Indicando que entre as doses, a menor, proporcionou resultados mais vantajosos para a produção de mudas orgânicas de tomateiro.

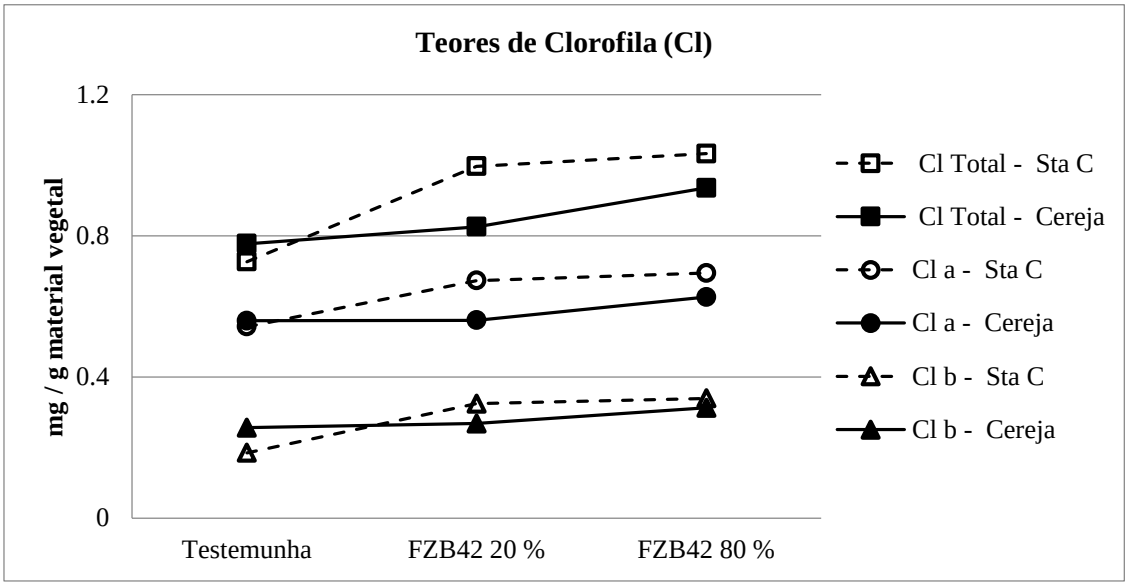

Figura 2. Teores de clorofila mensurados ao final da produção de mudas de tomateiro. Cultivar Santa Clara (Sta C) representada pela linha pontilhada e cultivar Cereja representada por linha contínua.

A variável mais responsiva à inoculação, dentre as testadas, foi a área da parte aérea. Nesta variável, a cultivar Santa Clara respondeu positivamente com mais intensidade na menor dose de FZB42, mostrando aumento de 47,74%, enquanto a 'Cereja', apresentou aumento de 15,54 %, ambos comparados à testemunha. Esta diferença indica resposta variável em função do genótipo, uma vez que as cultivares pertencem a grupos distintos. Interações entre bactérias e genótipos foram relatados por outros autores. Lemos *et al*. (2013), verificaram entre cinco cultivares de trigo com inoculação de *A. brasilense*, apenas uma se destacou, enquanto Ferreira *et al*. (2014), verificaram que entre seis cultivares de arroz, apenas uma apresentou interação

com diversas estirpes bacterianas, com diferença significativa em relação ao crescimento da parte aérea.

3.4 Conclusões

A bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, produtora de compostos indólicos e sideróforos, aumentou os teores clorofila *a*, *b* e totais e promoveu o crescimento da parte aérea de mudas de tomateiro das cultivares Santa Clara e Cereja, na dose de 20% da solução inoculante, correspondendo a $1{,}6x10^5$ bactérias/semente.

Agradecimentos

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo auxílio financeiro e à Omex® Agrifluids do Brasil Ltda, por ceder a cepa da bactéria.

3.5 Referências Bibliográficas

Brasil, Ministério da Agricultura. (2009) - *RAS Regras para análise de sementes*. Brasília: Departamento Nacional de Produção Vegetal, 399 p.

Carvalho, J.L. e Pagliuca, L.G. (2007) - Tomate: Um mercado que não para de crescer globalmente. *Revista Hortifruti Brasil*, vol. 58, p. 6-14.

Cassán, F.; Maiale, S.; Masciarelli, O.; Vidal, A.; Luna, V. e Ruiz, O. (2009) - Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *European Journal of Soil Biology*, vol. 45, p. 12-19.

Chen, X.H.; Koumoutsi, A.; Scholz, R.; Eisenreich, A.; Schneider, K.; Heinemeyer, I.; Morgenstern, B.; Voss, B.; Hess, W.R.; Reva, O.; Junge, H.; Voigt, B.; Jungblut, P.R.; Vater, J.; Süssmuth, R.; Liesegang, H.; Strittmatter, A.; Gottschalk, G. e Borriss, R. (2007) - Comparative analysis of the complete genome sequence of the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nature Biotechnology*, vol. 25, p. 1007-1014.

Compant, S.; Clément, C. e Sessitsch, A. (2010) - Plant growth-promoting bacteria in the rhizo and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, vol. 42, n. 5, p. 669-678.

Domenech, J.; Reddy, M.S.; Kloepper, J.W.; Ramos, B. e Gutierrez-Mañero, J. (2006) - Combined application of the biological product LS213 with *Bacillus*, *Pseudomonas* or

Chryseobacterium for growth promotion and biological control of soil-borne diseases in pepper and tomato. *BioControl*, vol. 51, p. 245-258.

Ferreira, E.P.B.; Knupp, A.M. e Martin-Didonet, C.C.C. (2014) - Crescimento de cultivares de arroz (*Oryza sativa* L.) influenciado pela inoculação com bactérias promotoras de crescimento de plantas. *Bioscience Journal*, vol. 30, n. 3, p. 655-665.

Food and Agriculture Organization of United Nations - FAOSTAT. (2010) - Database Results [Online]. [citado 2012-12-02]. Disponível em: <http://apps.fao.org>.

Food and Agriculture Organization of United Nations - FAOSTAT. (2011) - Food and Agricultural commodities production [Online]. [citado 2012-12-06]. Disponível em: <http://www.faostat.fao.org>.

Gül, A.; Kidoglu, F.; Tüzel, Y. e Tüzel, I. H. (2008) - Effects of nutrition and *Bacillus amyloliquefaciens* on tomato (*Solanum lycopersicum* L.) growing in perlite. *Spanish Journal of Agricultural Research*, vol. 6, n. 3, p. 422-429.

Harthmann, O.E.L.; Mógor, Á.F.; Wordell Filho, J.A.; Luz, W.C. e Biasi, L.A. (2009) - Tratamento de sementes com rizobactérias na produção de cebola. *Ciência Rural*, vol. 39, n. 9, p. 2533-2538.

Harthmann, O.E.L.; Mógor, Á.F.; Wordell Filho, J.A. e Luz, W.C. (2010) - Rizobactérias no crescimento e na produtividade da cebola. *Ciência Rural*, vol. 40, n. 2, p. 462-465.

Idris, E.E.S.; Bochow, H.; Ross, H. e Borriss, R. (2004) - Use of *Bacillus subtilis* as biocontrol agent. VI. Phytohormone like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. *Journal of Plant Disease and Plant Protection*, vol. 111, p. 583-597.

Jurkevitch, E.; Chen, Y. e Hadar, Y. (1988) - Involvement of bacterial siderophores in the remedy of lime-induced chlorosis in peanut. *Soil Science Society of America Journal*, vol. 52, n. 4, p. 1032-1037.

Khan, A.; Geetha, R.; Akolkar, A.; Pandya, A.; Archana, G. e Desai, A.J. (2006) - Differential cross-utilization of heterologous siderophores by nodule bacteria of *Cajanus Cajan* and its possible role in growth under iron-limited conditions. *Applied Soil Ecology*, vol. 34, n. 1, p. 19-26.

Krebs, B.; Höding, B.; Kübart, S.M.; Workie, A.; Junge, H.; Schmiedeknecht, G.; Grosch, R.; Bochow, H. e Hevesi, M. (1998) - Use of *Bacillus subtlis* as biocontrol agent activities and characterization of *Bacillus subtilis* strains. *Journal of Plant Diseases and Protection*, vol. 105, p. 181-197.

Lemos, J.M.; Guimarães, V.F.; Vendruscolo, L.C.G.; Santos, M.F. e Offemann, L.C. (2013) - Resposta de cultivares de trigo à inoculação de sementes com *Azospirillum brasilense*, e à adubação nitrogenada em cobertura. *Científica*, vol. 41, n. 2, p. 189-198.

Lichtenthaler, H.K. (1987) - Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, vol. 148, p. 350-382.

Lim, J.H. e Kim, S.D. (2009) - Synergistic plant growth promotion by the indigenous auxins-producing PGPR *Bacillus subtilis* AH18 and *Bacillus licheniforims* K11. *Journal of the Korean Society for Applied Biological Chemistry*, vol. 52, n. 5, p. 531-538.

Mena-Violante, H.G. e Olalde-Portugal, V. (2007) - Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. *Scientia Horticulturae*, vol. 113, p. 103-106.

Miché, L.; Bouillant, M.L.; Rohr, R.; Sallé, G. e Bally R. (2000) - Physiological and cytological studies on the inhibition of striga seed germination by the plant growth-promoting bacterium *Azospirillum brasilense*. *European Journal of Plant Pathology*, vol. 106, n. 4, p. 347-351.

Minami, K. (1995) - *Fisiologia da produção de mudas*. São Paulo: T. A. Queiroz, 129 p.

Patten, C.L. e Glick, B.R. (1996) - Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, vol. 42, p. 207-230.

Powell, K.A.; Faull, J.L. e Renwick, A. (1990) - The commercial and regulatory challenge. In: HORNY, D. (ed) *Biological control of soil borne plant pathogens*, CAB International Wallingford, United Kingdom, p. 445-463.

Rodrigues Neto, J.; Malavolta Júnior, V.A. e Victor, O. (1986) - Meio simples para isolamento e cultivo de *Xantomonas campestres* pv. *citri* tipo B. *Summa Phytopathologica*, vol. 12, p. 16.

Sharma, A; Johri, B.N; Sharma, A.K. e Glick B.R. (2003) - Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). *Soil Biology and Biochemistry*. vol. 35, n. 7, p. 887-894.

Silva, F.A.S. e Azevedo, C.A.V. (2002) - Versão do programa computacional Assistat para o sistema operacional Windows. *Revista Brasileira de Produtos Agroindustriais*, vol. 4, n. 1, p. 71-78.

Silva, J.R.C.; Souza, R.M.; Zacarone, A.B.; Silva, L.H.C.P. e Castro, M.A.S. (2008) - Bactérias endofíticas no controle e inibição *in vitro* de *Pseudomonas syringae* pv *tomato*, agente da pinta bacteriana do tomateiro. *Ciência e Agrotecnologia*, vol. 32, n. 4, p. 1062-1072.

Schwyn, B. e Neilands J. B. (1987) - Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, vol. 160, p. 47-56.

Schlindwein, G.; Vargas, L.K.; Lisboa, B.B.; Azambuja, A.C.; Granada, C.E.; Gabiatti, N.C.; Prates, F. e Stumpf, R. (2008) - Influência da inoculação de rizóbios sobre a germinação e o vigor de plântulas de alface. *Ciência Rural*, vol. 38, n. 3, p. 658-664.

Silveira, E.B.; Rodrigues, V.J.L.B.; Gomes, A.M.A.; Mariano, R.L.R. e Mesquita, J.C.P. (2002) - Pó de coco como substrato para produção de mudas de tomateiro. *Horticultura Brasileira*, vol. 20, p. 211-216.

Souza, J.L. e Resende, P.L. (2006) - *Manual de horticultura orgânica*. 2. ed. Viçosa: Aprenda Fácil, 843 p.

Szilagyi-Zecchin V.J.; Ikeda, A.C.; Hungria, M.; Adamoski, D.; Kava-Cordeiro, V.; Glienke, C. e Galli-Terasawa, L.V. (2014) - Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. *Applied Microbiology and Biotechnology Express*, vol. 4, n. 26, p.1-9.

Taiz, L. e Zeiger, E. (2004) - *Fisiologia vegetal*. Porto Alegre: Artmed, 719 p.

Toledo, D.S.; Costa, C.A.; Bacci, L.; Fernandes, L.A. e Souza, M.F. (2011) - Production and quality of tomato fruits under organic management. *Horticultura Brasileira,* vol. 29, p. 253-257.

4 CAPÍTULO II – THE PLANT DEVELOPMENT AND ALTERATIONS ON METABOLISM OF SUGARS AND PROTEINS IN TOMATO CULTIVARS BY THE INOCULATION OF DIFFERENT DOSES OF *Bacillus amyloliquefaciens*

Abstract
The use of plant-growth-promoting bacteria could contribute to sustainability of agriculture. Metabolic and morphometric variables, and its correlation with initial growth of two tomato cultivars was accessed in this study, aiming evaluate the effect of seeds inoculation with *Bacillus amyloliquefaciens* subs. *plantarum* FZB42, using two doses: $6x10^{10}$ CFU mL^{-1} (*Ba*10); and $2.4x10^{11}$ CFU mL^{-1} (*Ba*11) of inoculum, and a control with only distilled water (*Ba*0). The inoculation provided roots growth increments at different rates, varying from 14.56% (total length at 'Santa Clara I-530' cultivar) to 196.13% (root volume at 'Cherry 261' cultivar). While the aerial part increments varied from 12.06% (leaves area at 'Santa Clara I-530'), to 88.88% (dry matter at 'Cherry 261'). Increases of the root system at *Ba*10 reflected on increases in plant height and fresh matter weight of the aerial part. Metabolites concentration (sugars and soluble proteins) was closely related to leaves expansion according to cultivar response and doses. The *Ba*10 ($6x10^{10}$ CFU mL^{-1}) showed significant plant growth promotion effect on two cultivars of tomato initial growth, being promising for future works.

Key words: plant growth promotion bacteria, inoculation, bio-fertilizer, metabolites, soluble sugar, soluble protein.

Desarrollo de la planta y las alteraciones en el metabolismo de los azúcares y proteínas en el variedades cultivadas de tomate por la inoculación de diferentes dosis de Bacillus amyloliquefaciens

Resumem
El uso de bacterias promotoras del crecimiento de planta puede contribuir a la sostenibilidad de la agricultura. Metabólicos y variables morfométricas, y su correlación con el crecimiento inicial de los cultivares de tomate fue analisado en este estudio, con el objetivo de evaluar el efecto de la inoculación de semillas con Bacillus amyloliquefaciens *subs.* plantarum *FZB42, usando dos dosis:* $6x10^{10}$ UFC mL^{-1} (*Ba*10); and $2.4x10^{11}$ UFC mL^{-1} (*Ba*11) de *solución inoculante, y un control com agua destilada (Ba*0*). La inoculación he proporcionado incrementos de crecimiento en las raíces en proporciones diferentes, que van desde 14,56% (longitud total en la variedad 'Santa Clara I-530') a 196.13% (volumen en el cultivar 'Cherry 261'). Mientras que los incrementos de parte aérea varió de 12,06% (area de las hojas de 'Santa Clara I-530'), al 88.88% (materia seca de 'Cherry 261'). Los aumentos del sistema radicular en* Ba*20 se refleja en aumentos en la altura y peso de materia fresca de la parte aérea. Una concentración de metabolitos (azúcares y proteínas solubles) estaba estrechamente relacionada con la expansión de las hojas de acuerdo con la respuesta del cultivar y dosis. El*

Ba*10 (*6x10^{10} UFC mL^{-1}*) mostró efecto significativo de la promoción del crecimiento vegetal en dos cultivares de tomate en crecimiento inicial, siendo prometedor para futuros trabajos.*

Palabras clave: *bacterias promotoras del crecimiento vegetal, inoculación, bio-fertilizantes, metabolitos, azúcar soluble, proteínas solubles.*

4.1 Introduction

Bacteria able to increase plant growth have been tested for their possible contribution to become more sustainable the agriculture. Among them, *Bacillus* stands out, *ie*: *B. subtilis* promoted lettuce (*Lactuca sativa*) growth (Arkhipova *et al.,* 2005); *B. cereus, B. macroides* and *B. pumilus* stimulated red pepper (*Capsicum annum*) plants development (Joo *et al.,* 2004), and *B. amyloliquefaciens* increased tomato seedlings growth by seeds inoculation related to bacteria auxin production capacity (Szilagyi-Zecchin *et al.,* 2015a).

Some bacteria, besides producing and providing phytohormones, may metabolize hormone precursors. Hormone concentration is fundamental for regulation of various physiological processes, and modification of these levels by microorganisms may lead to variations of growth and development characteristics of the treated plants (Ahmed and Hasnain, 2010). In addition, seeds inoculation with growth promoting bacteria may causes changes on metabolites production of the primary metabolism, as carbohydrates, proteins and amino acids (Ravikumar *et al.*, 2014; Kang *et al.*, 2014).

Tomato (*Solanum lycopersicum* L.) is cultivated all over the world, has great economic and social relevance, because besides its consumption *in nature*, is consumed in many industrialized forms like juice, sauce, paste and dehydrated. The Brazil stands out with a production of approximately 3.8 million tonnes, placing it in 8th position of world production in 2012 (FAO, 2015). Therefore, assess the effect of inoculation of plant growth promoting bacteria on tomato growth, could contribute for sustainability of tomato production.

It was thus, the aim of the present work to verify biometric and biochemical alterations in two tomato cultivars with seeds inoculated with *Bacillus amyloliquefaciens* subs. *plantarum* FZB42 bacteria, to identify their potential as inoculant or bio-fertilizer.

4.2 Materials and Methods

Two tomato cultivars were used, ‘Santa Clara I-530’ (Isla®) and ‘Cherry 261’ (Isla®), with a cycle of respectively 110 and 90 days, both with undetermined growth rate.

Inoculation took place using *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 (Omex® Agrifluids do Brasil Ltda), stocked as 10A6 strain in the *Bacillus* Genetic Stock Center (BGSC, Ohio, U.S.A.).

The experiment was conducted at the Federal University of Paraná (UFPR), in greenhouse located at the Organic Production Research Area.

Seeds had not received chemical treatment were inoculated with bacteria solution, at $6x10^{10}$ CFU mL^{-1} (*Ba*10) and $2.4x10^{11}$ CFU mL^{-1} (*Ba*11) in the proportion of 320 uL g^{-1} of seed, and the control was inoculated with distilled water. Next, seeds were left drying in the shade, on paper towels, and then immediately sown.

Seeds were sown in expanded polystyrene trays, with 200 cells filled with composted bird bedding mixture (Provaso®) and pinus composted bark (Vida Verde®) in 0.5:3.5 w/w proportion. One seed per cell was placed, at 1 cm depth. A micro-aspersion irrigation system, with timer, worked during the day with a time interval of 3 minutes each two hours.

Seedlings were transferred to plastic vessels of 2 L, 30 days after sown, filled with the same substrate used for their production. One plant per vessel was maintained in the greenhouse for 30 days.

The experimental design was completely randomized with 2 x 3 factorial scheme (tomato cultivars x bacteria dose and control), where each treatment consisted in seven replications, totalizing 28 plants per treatment.

4.2.1 Morphometric analyses

The following variables were evaluated at 60 days after sowing (DAS): area of leaves (cm^2), total root length – length of all roots summarized – (cm); volume of roots – length and thickness of roots (cm^3); and volume of the aerial part – area of leaves and their thickness – (cm^3), using the computational software WinRhizo®, coupled to an LA1600 Scanner (Règent Instruments Inc®.- Canadá).

They were also evaluated at 60 DAS fresh matter weight of the aerial part and roots (g); dry matter weight of the aerial part and roots (g), obtained by drying in a forced ventilation oven at 60°C until constant weight was reached, and then weighting with an analytical precision scale; diameter of the stem (cm) at the insertion point of the first leaf from base; height of the plant from base to the insertion point of the last leaf; number of leaves and number of flower buds per plant.

The specific leaf area (SLA) was calculated by dividing the area of leaves (cm^2) by the dry matter weight (g) of the same portion of leaf.

4.2.2 Biochemical analyses

Leaf samples were obtained from leaflets of the middle of the third and fourth leaf counting from the top, at 60 days after sowing Leaflets were collected between 9:00 and 10:00 a.m., and next were macerated with liquid nitrogen in a mortar, until obtaining a fine powder. Values were expressed in µg of metabolite per g of leaf fresh weight.

Chlorophylls and carotenoids were extracted according to Lichtenthaler (1987) with acetone 80% in distilled H_2O, added with 0.1% of $CaCO_3$ (w/v) (Pompelli et al. 2013). Readings were made at 663, 647 and 470 nm. Equations described by Lichtenthaler and Buschmann (2001) were applied.

Reducing sugars were determined according to Miller (1959), and total sugars according to Maldonade *et al.* (2013). The standard curve was obtained with glycose at 1 mg mL^{-1} (5.5 mM), with values between 50 and 800 µg mL^{-1}. Non-reducing sugars were calculated subtracting reducing sugars from total sugars.

Extraction of soluble proteins was performed according to Du *et al.* (2010), and colorimetric reaction performed according to Bradford (1976). The standard curve was built with bovine serum albumin (BSA) at 0.2% (w/v), with values between 28 and 140 µg mL^{-1}.

4.2.3 Statistic

Data were submitted to ANOVA, and means were compared at 5 and 1% of significance by Scott-Knott test. A Pearson's correlation test was also performed between all the described variables. The software Assistat 7.7 Beta (Silva and Azevedo, 2002) was used for statistical analyses.

4.3 Results and Discussion

4.3.1 Morphometric analyses

There was interaction between doses and cultivars for all the variables referred to the roots (Table 1). The two doses promoted volume increases of around three times in 'Cherry 261' (Ch261), while in 'Santa Clara I-530 (SC530) there were decreases when inoculated with the *Ba*11. Total root length increased twice in 'Ch261' under both bacteria treatments, and 'SC530' only presented increased root length at *Ba*10 inoculation.

As the roots volume and length, the values of roots fresh and dry matter showed differences between cultivars, highlighting *Ba*10 as the dose which promoted the highest increases. Plants of 'SC530' inoculated with *Ba*11 showed less fresh and dry matter when compared to control, indicating high sensibility to the dose. In other hand, the two doses in 'Ch261' showed twice weight gains compared to control.

The promotion on root growth related to *Bacillus* strains inoculation was also reported by Ahmed and Hasnain (2010) in *Solanum tuberosum* with increases from 42 to 75% in root length; by Kang *et al.* (2014) using *Bacillus megaterium* in mustard plants with increases on root length of 21.79%; and by Mena-Violante and Olalde-Portugal (2007), in which tomato plants inoculated with *B. subtilis* presented root dry matter and roots length values increased respectively 30.09% and 16.9% at 50 days after transplantation. Szilagyi-Zecchin *et al.* (2015b) comparing bacterial strains, verified that *Bacillus* sp. promoted 65.1% of increases on root length of corn plants 30 days after inoculation, relating this effect to auxin released by *Bacillus* sp.

The increases on roots presented in this study, are probable related to auxinic stimulation provided by *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, since this strain can produce indolic compounds (Szilagyi-Zecchin *et al.,* 2015a). In plants, auxin (AIA) plays a fundamental role in cell elongation and induces roots development (Phillips *et al.,* 2011).

The microbial auxin is not only synthesized and released, but also can entering in the roots host cells in sufficient quantity to modify normal plant growth and development (Sukumar *et al*., 2013). Therefore, the differences between Ch261 and SC530 regarding to the roots growth could be related to the microbial indolic compounds release. As the bacterial AIA stimulates increases on roots growth if it stays within an ideal concentration, also the bacterial IAA can inhibits plant growth (Duca *et al*., 2014), depending the amount and the sensitivity of the plant tissue (Ali *et al.,* 2010; Spaepen *et al.,* 2007), as at the roots growth inhibition found on SC530 inoculated with *Ba*11.

There was no interaction between cultivars and doses regarding to the stem diameter and specific leaf area (SLA). The cultivars showed differences between them, while the doses no (Table 1).

All the other variables of the aerial part of plants (number of leaves, area of leaves, plant height and fresh and dry matter weight) showed interaction between doses and cultivars.

Number of leaves was lower in SC530 inoculated with *Ba*11 and not differed from control in *Ba*10. In Ch261 inoculated with both doses, was found the opposite, showing about

3 leaves more. The effect of *Bacillus* strains was reported by Ahmed and Hasnain (2010) increasing number of *Solanum tuberosum* leaves around 33%.

On average, at Ch261 the control presented 4.14 mature flower buds (totally developed buds, open or partially open), while plants inoculated with *Ba*10 and *Ba*11 had more than two fold flowers, respectively 10.71 and 11.5 (Figure 1). Thereby, according to the Solenaceae phenological growth stages identification key (Feller *et al.,* 1995), control was at growth stage 5 as to the appearance of flowers, while inoculated plants were already in growth stage 6, with the plants fully flowering, showing open flowers, indicating that inoculation accelerated the plants development.

Regarding to the plant height, SC530 plants at *Ba*11 were shorter than control, while Ch261 plants, instead, were taller than control when inoculated, as a probable response to the bacterial indole compounds that can promote or inhibits plant growth. The AIA produced by bacteria acts together with the endogenous supply of the plant. Thus, the impact of bacterial AIA are closely related to sensibility of the plant tissues (Ali *et al.,* 2010).

The leaves area of SC530 cultivar was increased with *Ba*10 inoculation, and decreased to around a half when inoculated with *Ba*11. Meanwhile, Ch261 practically doubled its leaves area under both doses. A leaf area increasing effect by auxin producer *Bacillus* sp strain was found by Szilagyi-Zecchin *et al.* (2015b) on inoculated corn plants, showing 39.4% highest leaves area.

The aerial part dry and fresh matter weight showed the same reaction pattern: in SC530, *Ba*10 increased weight and *Ba*11 decreased it; in Ch261, significant increases were observed mainly in dry matter weight, under both doses. In general, SC530 inoculated with *Ba*11 presented almost on all the morphometric variables (except stem diameter and root length) lower values than the control (Table 1), demonstrating that this was an excessive dose, causing reduction of the plant growth. Bacteria concentration also reflects the quantity of microbial AIA that the plant was exposed to, and therefore determines if bacteria promote or inhibit plant growth (Duca *et al.,* 2014). However, other factors cannot be ruled out, as other metabolites besides auxin, which contribute to plant growth inhibition in high concentration inoculants (Spaepen *et al.,* 2007).

4.3.2 Biochemical analyses

The pigments (chlorophylls and carotenoids) did not show variation because of inoculation (data not shown).

There was no interaction between doses and cultivars for sugars, but differences between doses were observed by the test of means (Table 2). SC530 leaves at both doses showed reduction on total sugars content. In Ch261 non-reducing sugars were not differed, while inoculated SC530 plants had their values decreased according to the dose.

The decrease of reducing sugars seen in Ch261 at *Ba*10 (Table 2) could be related to the use of these carbohydrates to build cell walls, which can be determined by the increase of leaf volume (Table 1). In the same way, leaves of SC530 at *Ba*11 had more reducing sugars than the other treatments, showing in this case, the lowest area and volume of the leaves. Therefore, the reducing sugar content is related to the leaves expansion. It is corroborated by the negative correlations observed between morphometric and biochemical variables. When smaller are the leaves area and dry matter weight of the aerial part, higher are the carbohydrates concentrations, regarding to the minor expansion of leaves (Table 3). This reduction in leaf development may be linked to excess auxin imposed by bacteria, these data are corroborated by other work which made application of exogenous auxin at high concentrations in common bean (*Phaseolus vulgaris*) and *Arabdopsis,* also showing reduction of leaf area of these species (Keller *et al.*, 2004).

Total soluble protein concentrations (Table 2) are also intimately bound to leaves expansion and directly related to mass quantity, because metabolites are expresses in $\mu g\ g^{-1}$ of fresh matter weight. Therefore, it is possible to observe that in treatments where there was greater development of the aerial part (higher weight) (Ch261 *Ba*10 and *Ba*11) less proteins were detected, and at the other hand, in the treatment with minor plant development (SC530 *Ba*11), there is more protein per g of matter. At last, where lower are the leaves area and dry matter weight of the aerial part, higher is the protein concentration (Table 3).

Linear correlation analyses, comparing root system and aerial part aspects, showed correlations with positive values of the Pearson's coefficients for both cultivars when inoculated with *Ba*10 (Table 3). Therefore, increases of the root system with *Ba*10 reflected on increases in plant height and fresh matter weight of the aerial part. With *Ba*11 dose, Ch261 kept presenting positive correlation between the same above mentioned variables. On the other hand, SC530 presented negative correlations, thus, the growth of aerial part not reflected the roots growth.

Increases in the root system and aerial part components were verified on both cultivars by inoculation of *Bacillus amyloliquefaciens* FZB42 at the dose *Ba*10. Those increases provided different percentages in the root system increments, which varied from 14.56% (total length,

SC530), to 196.13% (root volume, Ch261), while the aerial part varied from 12.06% (leaves area, SC530), to 88.88% (dry matter, Ch261). Those gains highlight the positive effect on plants growth; so much that positive linear correlations confirm these growth stimulations on the aerial part and roots, for both cultivars at *Ba*10.

Besides all these aspects above discussed, it must be considered that the experiment was conducted with materials characterized by very distinct tomato genotypes, and the difference in growth cycle of these genotypes may have influenced the reaction to inoculation. Ch261 has a shorter cycle (90 days until start to the harvest) and already had flower buds, while SC530 has a 110 days cycle until start the harvest, and had not flourished yet when the experiment ended. Therefore, they were in different physiological statuses.

4.4 Conclusions

The inoculation with *Bacillus amyloliquefaciens* FZB42, promoted tomato plants growth in both tested cultivars. The *Ba*10 dose provided better increments for aerial part and roots. SC530 is more sensible to the inoculation dose, at *Ba*11 presented less development (aerial part and roots) when compared to control. The results indicated that the inoculation of *Bacillus amyloliquefaciens* FZB42 at $6x10^{10}$ CFU mL^{-1} (*Ba*10) is a promising tool to contribute to the sustainability of tomato production, promoting initial plant growth, also stimulating future studies aimed to verify the action of these bacteria on yield.

Acknowledgement

Our thanks go to Omex® Agrifluids of Brasil Ltda, for providing the strain of bacteria. This work was supported by Coordination for the Improvement of Higher Education Personnel (CAPES), process: 308029/2015-1, and by the National Counsel of Technological and Scientific Development (CNPq), process: 308029/2015-1 –PQ2.

4.5 Literature Cited

Ahmed, A.; Hasnain, S. 2010. Auxin-producing *Bacillus* sp.: auxin quantification and effect on the growth of *Solanum tuberosum*. *Pure and Applied Chemistry*, 82: 313-319.

Ali, B.; Sabri, A.N.; Hasnain, S. 2010. Rhizobacterial potential to alter auxin content and growth of *Vigna radiata* (L.). *World Journal of Microbiology and Biotechnology*, 26: 1379-1384.

Arkhipova, T.N.; Veselov, S.U.; Melentiev, A.I.; Martynenko, E.V.; Kudoyarova, G.R. 2005. Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. *Plant and Soil*, 272(1-2): 201-209.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1): 248-254.

Du, C.X.; Fan, H.F.; Guo, S.R.; Tezuka, T. 2010. Applying spermidine for differential responses of antioxidant enzymes in cucumber subjected to short-term salinity. *Journal of American Society of Horticulture Science*, 135(1):18-24.

Duca, D.; Lorv, J.; Patten, C.L.; Rose, D.; Glick, B.R. 2014. Indole-3-acetic acid in plant–microbe interactions. *Antonie Van Leeuwenhoek*, 106(1): 85-125.

Feller, C.; Bleiholder, H.; Buhr, L.; Hack, H.; Hess, M.; Klose, R.; Meier, U.; Stauss, R.; Boom, T. Van Den; Weber, E. 1995. Phänologische Entwicklungsstadien von Gemüsepflanzen: II. Fruchtgemüse und Hülsenfrüchte. *Nachrichtenbl DeutPflanzenschutzd*, 47: 217-232.

FAO - Food and Agriculture Organization of the United Nations. Data base Faostat – Agriculture, 2013. Roma: FAO, 2015. Available in: http://faostat.fao.org/site/339/default.aspx. Accessed in: 20/December/2015.

Joo, G.J.; Kim, Y.M.; Lee, I.J.; Song, K.S.; Rhee, I.K. 2004. Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. *Biotechnology Letters*, 26(6): 487-491.

Kang, S.M.; Radhakrishnan, R.; You, Y.H.; Joo, G.J.; Lee, I.J.; Lee, K.E.; Kim, J.H. 2014. Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. *Indian Journal of Microbiology*, 54(4): 427-433.

Keller, C.P.; Stahlberg, R.; Barkawi, L.S.; Cohen, J.D. 2004. Long-term inhibition by auxin of leaf blade expansion in bean and Arabidopsis. *Plant Physiology*, 134(3): 1217-1226.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148: 350-382.

Lichtenthaler, H.K.; Buschmann, C. 2001. Chlorophylls and carotenoids: Measurement and characterization by UV–VIS spectroscopy. In: Current protocols in food analytical chemistry (CPFA). Wrolstad, R.E.; Acree, T.E.; Na, H.; Decker, E.A.;, Penner, M.H.; Reid, D.S.; Schwartz, S.J.; Shoemaker, C.F.; Sporns, P. (ed.s).. John Wiley and Sons, New York; USA. . Pp. F4.3.1–F4.3.8.

Maldonade, I.R.; Carvaho, P.G.B.; Ferreira, N.A. 2013. Protocolo para a Determinação de Açucares Totais em Hortaliças pelo Método de DNS. EMBRAPA. Brasília, Brazil. ComunicadoTecncio 85. 4p.

Mena-Violante, H.G.; Olalde-Portugal, V. 2007. Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. *Scientia Horticulturae*, 113: 103-106.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagente for determination of reducing sugar. *Analytical Chemistry*, 31(3): 426-428.

Phillips, K.A.; Skirpan, A.L.; Liu, X.; Christensen, A.; Slewinski, T.L.; Hudson, C.; Barazesha, S.; Cohenb, J.D.; Malcomberc, S.; McSteen, P. 2011. Vanishing tassel2 encodes a grassspecific tryptophan aminotransferase required for vegetative and reproductive development in maize. *Plant and Cell*, 23: 550-566.

Pompelli, M.F.; França, S.C.; Tigre, R.C.; Oliveira, M.T.; Sacilot, M.; Pereira, E.C. 2013. Spectrophotometric determinations of chloroplastidic pigments in acetone, ethanol and dimethylsulphoxide. *Revista Brasileira de Biociências*,11(1): 52-58.

Ravikumar, S.; Shanthy, S.; Kalaiarasi, A.; Sumaya, M. 2014. The Biofertilizer effect of halophilic phosphate solubilising bacteria on *Oryza sativa*. *Middle-East Journal of Scientific Research*, 19(10): 1406-1411.

Silva, F.A.Z.; Azevedo, C.A.V. 2002. Versão do programa computacional Assistat para o sistema operacional Windows. *Revista Brasileira de Produtos Agroindustriais*, 4(1): 71-78.

Spaepen, S.; Vanderleyden, J.; Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews*, 31: 425-448.

Sukumar, P.; Legue, V.; Vayssieres, A.; Martin, F.; Tuskan, G.A.; Kalluri, U.C. 2013. Involvement of auxin pathways in modulating root architecture during beneficial plant–microorganism interactions. *Plant, Cell and Environment*, 36(5): 909-919.

Szilagyi-Zecchin, V.J.; Mógor, Á.F.; Ruaro, L.; Röder, C. 2015a. Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 em cultura orgânica. *Revista de Ciências Agrárias*, 38(1): 26-33.

Szilagyi-Zecchin, V.J.; Klosowski, A.C.; Ikeda, A.C.; Hungria, M.; Galli-Terasawa, L.V.; Kava-Cordeiro, V.; Glienke, C.; Mógor, A.F. 2015b. Potential inoculant strains of Brazilian endophytic bacteria for maize (*Zea mays* L.) growth promotion. *International Journal of Agronomy and Agricultural Research*, 7(4): 128-134.

Table 1. Morphometric analyses in plants of two tomato cultivars, 60 days after sowing, inoculated with *Bacillus amyloliquefaciens* FZB42 (*Ba*) in a greenhouse trial. (SLA) specific leaf area, (GA) general average, (CV) coefficient of variation.

	SC530	**Ch261**	**SC530**	**Ch261**	**SC530**	**Ch261**	**SC530**	**Ch261**
				ROOT				
	Volume (cm^3)		Length (cm)		Fresh weight (g)		Dry weight (g)	
Ba 0	1.61^{aA}	1.74^{cA}	625.29^{bB}	402.62^{cB}	1.76^{bA}	1.56^{cA}	0.08^{aA}	0.05^{cB}
Ba 10	1.80^{aB}	5.17^{aA}	716.36^{aB}	904.54^{aA}	2.50^{aB}	4.51^{aA}	0.10^{aB}	0.13^{aA}
Ba 11	1.21^{bB}	4.42^{bA}	594.54^{bB}	809.52^{bA}	1.20^{cB}	3.67^{bA}	0.06^{bB}	0.11^{bA}
GA	1.54	4.42	645.40	705.56	1.82	3.25	0.08	0.10
CV%	10.49		7.08		15.58		16.3	
				ANOVA				
Doses (D)	**		**		**		**	
Cultivars (C)	**		**		**		**	
D x C	**		**		**		**	
				SHOOT				
	SLA ($cm^2\ g^{-1}$)		Stem diameter (cm)		Plant height (cm)		Leaf number	
Ba 0	738.02	1003.66	3.09	3.74	26.41^{aA}	17.69^{bB}	6.65^{aB}	7.48^{bA}
Ba 10	847.48	1123.91	3.11	3.87	27.73^{aA}	24.97^{aA}	6.65^{aB}	10.15^{aA}
Ba 11	671.05	857.88	2.85	3.81	21.20^{bB}	25.07^{aA}	5.45^{bB}	10.33^{aA}
	753.67^{B}	993.61^{A}	3.02^{B}	3.81^{A}	25.12	22.58	4.24	9.32
CV%	34.23		9.02		11.27		6.92	
				ANOVA				
Doses (D)	ns		ns		**		**	
Cultivars (C)	*		*		**		**	
D x C	ns		ns		**		**	
	Leaf area (cm^2)		Leaf volume (cm^3)		Fresh weight (g)		Dry weight (g)	
Ba 0	612.56^{bA}	361.32^{cB}	13.69^{aA}	4.38^{bB}	9.25^{bB}	20.75^{bA}	0.83^{bA}	0.36^{bB}
Ba 10	686.46^{aA}	764.26^{aA}	14.00^{aA}	10.73^{aB}	11.67^{aB}	28.77^{aA}	0.81^{aA}	0.68^{aB}
Ba 11	382.50^{cA}	617.68^{bA}	6.83^{bA}	8.05^{aA}	5.62^{cB}	28.61^{aA}	0.57^{cB}	0.72^{aA}
	560.51	360.98	11.51	7.72	8.85	26.04	0.77	0.59
CV%	13.3		29.75		12.39		17.01	
				ANOVA				
Doses (D)	**		**		**		**	
Cultivars (C)	**		**		**		**	
D x C	**		**		**		**	

Note: Equal letters, uppercase between cultivars (line) and lowercase between doses of the same cultivar (column), do not differ by Scott-Knott test $p \leq 0.01$. ANOVA: (ns) not significant, (*) $p \leq 0.05$, (**) $p \leq 0.01$.

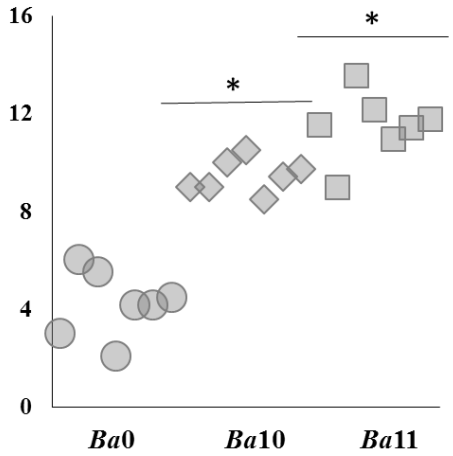

Figure 1. Flower bud number of 'Cherry 261' at 60 days after sowing. Geometric forms correspond to: circle = *Ba*0, lozenge = *Ba*10, and square *Ba*11. Values are means (n = 4 plants), (*) significative difference between treatments and control (*Ba*0) by Scott-Knott test $p \leq 0.01$.

Table 2. Biochemical analysis in leaves of two tomato cultivars, 60 days after sowing, inoculated with *Bacillus amyloliquefaciens* FZB42 (*Ba*) in a greenhouse trial. Values were expressed in μg of metabolites per g of fresh weight. (CV) Coefficient of variation. ANOVA: (C) cultivars and (D) doses.

	SC530	**Ch261**	**SC530**	**Ch261**	**SC530**	**Ch261**	**SC530**	**Ch261**
	Total soluble sugars		Reducing sugars		Non-reducing sugars		Total soluble proteins	
Ba 0	9036.36 ^{aA}	2660.61 ^{aB}	1177.78 ^{bA}	1143.43 ^{bA}	7858.58 ^{aA}	1517.17 ^{aB}	460.57 ^{bB}	599.14 ^{aA}
Ba 10	7939.39 ^{bA}	2436.36 ^{aB}	1060.61 ^{bA}	894.95 ^{cB}	6878.78 ^{bA}	1541.41 ^{aB}	462.00 ^{bA}	349.14 ^{bB}
Ba 11	7175.76 ^{bA}	2824.24 ^{aB}	2391.92 ^{aA}	1335.35 ^{aB}	4783.84 ^{cA}	1488.89 ^{aB}	687.71 ^{aA}	394.86 ^{bB}
CV %	11.06		8.27		12.95		9.67	
ANOVA								
C	**		**		**		**	
D	*		**		**		**	
C x D	*		**		**		**	

Note: Equal letters, uppercase between cultivars (line) and lowercase between doses of the same cultivar (column), do not differ by Scott-Knott test $p \leq 0.01$. ANOVA: (*) $p \leq 0.05$, (**) $p \leq 0.01$.

Table 3. Correlation analysis between morphometric characteristics and metabolites of two tomato cultivars, with 60 days after sowing, inoculated with *Bacillus amyloliquefaciens* FZB42 (*Ba*) in a greenhouse trial. (*p-value* > 0,0077).

Doses and Cultivars			r Pearson	*p-value*
***Ba* 10 - Santa Clara I-530**				
Root		**Shoot**		
length	x	height	0,9635	0,0364
length	x	fresh weight	0,9872	0,0128
***Ba* 11 - Santa Clara I-530**				
Root		**Shoot**		
length	x	leaves area	-0,7462	0,0537
length	x	dry weight	-0,9464	0,0513
Metabolites		**Shoot**		
total sugar	x	leaves area	-0,9575	0,0425
total sugar	x	dry weight	-0,9923	0,0077
reducing sugar	x	leaves area	-0,9575	0,0425
reducing sugar	x	dry weight	-0,9923	0,0077
non-reducing sugar	x	leaves area	-0,9575	0,0425
non-reducing sugar	x	dry weight	-0,9923	0,0077
protein	x	dry weight	-0,9909	0,0091
***Ba* 10 - Cherry 261**				
Root		**Shoot**		
length	x	height	0,9632	0,0367
dry weight	x	height	0,9726	0,0274
length	x	fresh weight	0,9511	0,0491
dry weight	x	fresh weight	0,9827	0,0173
***Ba* 11 - Cherry 261**				
Root		**Shoot**		
volume	x	leaves number	0,9575	0,0424
dry weight	x	leaves number	0,9513	0,0487

5 CAPÍTULO III – GROWTH AND BIOCHEMICAL CHANGES ON TOMATO SEEDLINGS INOCULATED WITH *Bacillus amyloliquefaciens*

Abstract
Looking for an sustainable approach on tomato seedlings production, this study evaluated the growth and biochemical changes on seedlings of two tomato cultivars whose seeds were inoculated with three concentrations of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, as follow: $1.5x10^9$ CFU mL^{-1} ($FZB42^9$); $6x10^{10}$ CFU mL^{-1} ($FZB42^{10}$); and $2.4x10^{11}$ CFU mL^{-1} ($FZB42^{11}$). The inoculation promoted increases of 11.89% on joint average of shoots and roots of the two cultivars at $FZB42^9$ and 39.52% at $FZB42^{10}$, and growth reduction on $FZB42^{11}$. The inoculation enhanced the photosynthetic apparatus. At the $FZB42^9$ and $FZB42^{10}$, the plants showed increases of the chlorophyll and, as a consequence of photosynthetic activity, it was accompanied by increases in total soluble proteins, soluble sugars and also the dry matter was increased on shoots and roots, and the area of aerial part of the seedlings of the two tomato cultivars, especially at $FZB42^{10}$. The bacteria capacity to release indolic compounds was characterized and this metabolite could acts in conjunction with the plant's endogenous indolic compounds. Depending the amount and the sensitivity of the plant tissue these compounds can promotes or inhibits plant growth. A larger shoot of inoculated plants, found in this study, allows a better photosynthetic rate, which implies in more soluble sugars to the growth or reserve for the following seedlings stages, contributing to facilitate a better establishment of plants on the field.

Keywords: plant growth promoting bacteria, chlorophylls, indolic compounds, soluble sugars, soluble proteins.

5.1 Introduction

Some species of bacteria can alter plant growth and development by producing plant growth regulating substances, like auxin. The bacterial hormones could causes changes on plant ontogeny, which are commonly associated with the increased grown (Lynch & Ho, 2005). Indole-3-acetic acid (IAA) is quantitatively the most abundant type of auxin that plants are able to synthesize, and the microorganisms can contribute to the plant auxin pool (Arkhipova, Veselov, Melentiev, Martynenko & Kudoyarova, 2005). Due to this ability, IAA producing bacteria have been used as inoculants to enhance the plant growth (Asghar, Zahir & Arshad, 2004).

Bacteria also can induce changes on plants metabolites, as the chlorophyll (Chauhan, Bagyaraj & Sharma, 2013), soluble sugars and soluble proteins on leaves (Vardharajula,

Zulfikar Ali, Grover, Reddy & Bandi, 2014), that contribute to the growth promotion (Szilagyi-Zecchin, Mógor, Ruaro & Röder, 2015a).

Bacillus are bacteria that have a positive influence on plant growth, (Szilagyi-Zecchin et al. 2014), and featuring feasibility to be formulated for commercial use (Ongena & Jacques, 2008). *Bacillus amyloliquefaciens* FZB42 the type strain for *B. amyloliquefaciens* subsp. *plantarum*, had the whole genome sequence determined in 2007 (Chen et al., 2007), as the first representative of gram-positive plant growth-promoting bacteria. This strain has important characteristics for agronomic use, previous researches shows that this bacteria could produce indolic compouds (Idris, Iglesias, Talon & Borriss, 2007; Szilagyi-Zecchin, Mógor, Ruaro & Röder, 2015a).

Accelerate the seedling growth in the nursery contributes to healthier and vigorous seedlings which in turn to facilitates better establishment of plants on the field (Thomas & Upreti, 2015). Therefore, as a tool for a sustainable tomato seedlings production, the aim of this study was to ascertain the growth of roots and shoots, and the biochemical changes on seedlings of two tomato cultivars grown in nursery with seeds inoculated with *Bacillus amyloliquefaciens* subsp. *plantarm* FZB42 at different concentrations. Furthermore, determine the strain capacity to release indole compounds.

5.2 Material and methods

The inoculation was performed with *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42, stocked as 10A6 strain in the *Bacillus* Genetic Stock Center (BGSC, Ohio, U.S.A.), provided by Omex® Agrifluids of Brasil Ltda.

Two tomato cultivars were used, 'Santa Cruz Kada Gigante' (Top Seed®) and 'Serato F1' (Top Seed®).

A bacterial cell suspension was incubated at 30°C for 24 hours at 150 rpm in Luria-Bertani (10 g tryptone, 10 g NaCl, and 5 g yeast extract per 1 L) broth. Then the suspension was centrifuged at 10.000 x *g* and re-suspended in saline solution (0.85% NaCl). The bacteria concentration was adjusted by serial dilutions, and the optical density (OD) values for the dilutions were measured with in spectrophotometer at 600 nm and together the plate counts to obtain colony forming unit (CFU) were made for each one. Calibration curve was drawn by plotting optical density OD versus viable number of bacteria (log CFU mL^{-1}). From curves

some concentration were choosen to compose the treatments inoculum: (Control) only distilled water ($FZB42^0$); $1.5x10^9$ CFU mL^{-1} ($FZB42^9$); $6x10^{10}$ CFU mL^{-1} ($FZB42^{10}$); and $2.4x10^{11}$ CFU mL^{-1} ($FZB42^{11}$). Seeds were inoculated in the proportion of 320 uL g^{-1} of seed. After inoculation, the seeds were left drying in the shade, on paper towels, and then immediately sown.

5.2.1 Greenhouse assay

The experiment was conducted at the Federal University of Paraná (UFPR), using a greenhouse located at the Organic Production Research Area of the same UFPR.

Seeds inoculated with bacteria solution according to the treatments without previous asepsis were sowed in expanded polystyrene trays, with 200 cells filled with substrate, composed by composted bird bedding mixture (Provaso®) and pinus composted bark (Vida Verde®) in 0.5:3.5 p/p proportion. One seed per cell was placed, at 1 cm depth. A micro-aspersion irrigation system, with timer, worked during the day with a time interval of 5 minutes each two hours.

The experimental design was completely randomized with 2 x 4 factorial scheme (tomato cultivars x bacteria concentrations and control), where each treatment consisted in four replications, totalizing 40 seedlings per treatment.

5.2.1.1 Morphometric analyses

Five days after planting (DAP) the emergence percentage of the plants were evaluated. Eight seedlings of each repetition with 35 DAP, were evaluated for: area of aerial part (cm^2), volume of roots (VR) – length and thickness of roots (cm^3), and roots thickness stratification at intervals of 0.5 mm (FVR) (cm^3), using WinRhizo® software coupled to an LA1600 Scanner (Règent Instruments Inc®., Canada).

Also the fresh matter weight of aerial part (FMa) and roots (FMr) (g) were measured; dry matter weight of aerial part (DMa) and roots (DMr) (g), obtained in a forced ventilation oven at 60°C until constant weight was reached.

5.2.1.2 Biochemical analyses

The samples were composed using all true leaves of the plants, of 10 plants of each treatments, with 35 DAP. The leaves were macerated with liquid nitrogen in a mortar, until obtaining a fine powder. Values of metabolites were expressed in µg per g of fresh leaves weight.

Chlorophylls and carotenoids were extracted according to Lichtenthaler (1987) with acetone 80% in distilled water, added with 0.1% of $CaCO_3$ (w v^{-1}) (Pompelli et al., 2013). Readings were made at 663, 647 and 470 nm in spectrophotometer. Equations described by Lichtenthaler and Buschmann (2001) were applied:

$$[\text{Cl } a] = 12{,}25 \times A663\text{ nm} - 2{,}79 \times A647\text{ nm}$$
$$[\text{Cl } b] = 21{,}50 \times A647\text{ nm} - 5{,}10 \times A663\text{ nm}$$
$$[\text{Cl } a + b] = 18{,}71 \times A647\text{ nm} + 7{,}15\ A663\text{ nm}$$
$$[\text{Car}] = (1000 \times 470\text{ nm}) - (1{,}82 \times \text{Cl } a) - (85{,}02 \times \text{Cl } b)/198$$

Total sugars extraction was performed with a 0.3 g of fresh matter on 2 mL of distilled water, and agitated by vortexing for 20 s. After centrifugued at 9335 x *g* for 15 min the sample supernatant was collected. The alyses of total sugars were determined with colorimetric reaction with 3.5-Dinitrosalicylic acid (DNS) (Miller, 1959). A samples aliquot of 1 mL was mixed with 1 mL of the DNS reagent and incubated in a boiling water bath for 15 min. After incubation added 1 mL of Rochelle salt (tartrate of sodium and potassium 40% w v^{-1}) and than cooling in ice bath for 10 min. The absorbance was measured in spectrophotometer at 540 nm. The standard curve for reducing and total sugars was obtained with glycose 5.5 mM, with values between 50 and 800 µg mL^{-1} that generated the equation:

$$y = 0.0033\ x - 0.101, \text{ with an } R^2 = 0.9947.$$

The extraction of soluble proteins was performed with 0.5 g of fresh matter in 1.5 mL of buffer, according to according to Du, Fan, Guo and Tezuka (2010) with modification: phosphate buffer pH 7.5 and 100 mM, with the addiction of 1 mM EDTA, 3 mM 1,4-dithiothreitol (DTT), 4% polyvinylpyrrolidone (PVP) (w v^{-1}) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The solution were homogeneized by vortexing for 10 s at low speed, and after centrifugued at 9,000 x *g* for 15 min. The supernatant was collected for measuring in spectrophotometer at 595 nm by the protein dye-binding method of Bradford (1976). The standard curve was built with bovine serum albumin (BSA) at 0.2% (w v^{-1}), with values between 27 and 139 µg mL^{-1} that generated the equation:

$$y = 0.0281\ x + 0.0153, \text{ with an } R^2 = 0.9954.$$

5.2.2 Colorimetric quantification of indolic compound production by bacteria

For the inoculum, a bacteria solution at a concentration of $1.5x10^9$ CFU mL^{-1} was used and 0.4 µL, was transferred to 4 mL of DYGS (Rodrigues Neto, Malavolta Jr & Victor, 1986) liquid medium supplemented/or not with 5 mM of L-tryptophan. The culture was incubated at 28°C, 120 rpm for until 3 days. After 24 and 72 hours of incubation, the culture was centrifuged at 9000 x *g* for 10 min.

The amount of indolic compounds per mL of culture was estimated adding 1 mL of culture supernatant to 1 mL of Salkowski's reagent (Gordon & Webber, 1951). After 30 minutes the color intensity was measured at 530 nm in spectrophotometer (Asghar, Zahir, Arshad & Khaliq, 2002). The auxin concentration was estimated using a standard curve prepared with known amounts of IAA (Sigma-Aldrich, USA).

5.2.3 Statistical analyses

Normality of data was verified by the Kolmogorov-Smirnov test, and homogeneity of variances by Bartlett test. Next, data were submitted to ANOVA, and means were compared at 5 and 1% of significance by Scott-Knott and Tukey test. The software Assistat 7.7 Beta (Silva & Azevedo, 2002) was used for statistical analyses.

5.3 Results

5.3.1 Greenhouse assay

5.3.1.1 Morphometric analyses

There was interaction between bacterial concentrations and tomato cultivars for all morphometric variables (Table 1), and cultivars differed in their responses in all evaluated aspects of root and shoot.

Was not observed statistical differences regarding to seedlings emergence percentage. The inoculated and no-inoculated on both cultivars, showed emergence around 90%.

Regarding to roots volume, Santa Cruz Kada Gigante (SC) showed increases at concentration of $FZB42^9$ and $FZB42^{10}$, but decrease at $FZB42^{11}$ (Figure 1A). The cultivar Serato (Se) also showed lower root volume at $FZB42^{11}$. Both cultivars had the same pattern of

response for roots dry weight in all doses, plants inoculated with FZB42^{10} had additions and FZB42^{11} had decreases (Figure 1B).

The area of seedlings aerial part (leaves and shoots) showed increments when inoculated with FZB42^{9} and FZB42^{10}. In other hand, inoculation with FZB42^{11} promoted significant area reduction, with mean less than the control (Figure 1C). The shoots dry mass was increased at FZB42^{10} on both cultivars, and as found for the area, FZB42^{11} causes a decreases. (Figure 1D).

The morphometric variables indicates that inoculation with FZB42^{9} and FZB42^{10} they acted on both cultivars on promoting the growth of shoots and roots. And FZB42^{11} limited the growth also compared to the control.

The thickness of the roots were presented in percentage, related to fractionated intervals of 0.5 mm (Figure 2). The SC inoculated with FZB42^{0} and FZB42^{9} had similar thickness roots. In FZB42^{10}, for the same cultivar, it was observed that 15% of roots belong to a thickness of 3.5 to 4.0 mm. But 'SC' with FZB42^{11} a largest number of fine roots was found, more than half of the roots (53.31%) with thickness less than 1.5 mm. 'Se' presented root thick patterns very similar between the control and the doses on average 38% of the roots measured up to 2 mm and 61.3% measured over 2 mm (Figure 2B).

5.3.1.2 Biochemical analyses

There was interaction between cultivars and FZB42 concentration for all foliar pigments (Table 1). The cultivars have different responses with each other for all pigments and bacterial concentration. Chlorophylls *a*, *b*, total, and carotenoids showed higher levels in 'SC' in all concentration of FZB42 tested (Figure 3A-D). Noteworthy is the FZB42^{11} concentration, which was higher than the others concentration tested. For 'Serato' the chlorophylls were high in FZB42^{9}, and carotenoids in FZB42^{11} (Figure 3A-D).

The total soluble sugars content showed interaction between cultivars and bacterial concentration. The 'SC' showed higher levels when inoculated with FZB42^{10} which was reduced at FZB42^{11} (Figure 4A). The 'Se' total soluble sugars was increased at FZB42^{9} and FZB42^{10}, and also decreased at FZB42^{11}.

The total soluble proteins show interaction between cultivars and bacterial concentration. The both cultivars shows that FZB42^{9} and FZB42^{10} has improved the protein content and FZB42^{11} reduced it. (Figure 4B).

5.3.2 Colorimetric quantification of indolic compound production by bacteria

At 24 hours after incubation of the bacterial solution, it was not possible to detect indolic compounds in a non supplemented L-tryptophan medium. In medium with 5 mM L-tryptophan, FZB42 produced 12.31 µg mL^{-1} of indolics compounds.

At 72 hours after incubation was determined in the unsupplemented L-tryptophan medium 2.45 µg mL^{-1} of indolic compounds, and at the supplemented medium we detected 11.65 µg mL^{-1}.

5.4 Discussion

The inoculation of *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 on tomato seeds, promoted changes in the vegetative development of seedlings, altering aerial part and roots growth. The results indicated that plant x bacteria interaction was variable according the bacteria concentration and tomato genotype, even as in work of Thomas and Upreti (2015) where inoculation with the same strain in different genotypes of tomato plants promoted specific responses for each interaction.

The increases found in vegetative growth of tomato plants, with 11.89% on joint average of shoots and roots at $FZB42^{9}$ ($1.5x10^{9}$ CFU mL^{-1}) and 39.52% at $FZB42^{10}$ ($6x10^{10}$ CFU mL^{-1}) can be attributed to the ability of *B. amyloliquefaciens* FZB42 to synthesize some metabolites, as the production of indolic compounds (Szilagyi-Zecchin et al., 2015a). Among the compounds produced by this strain, was detected by Idris, Bochow, Ross & Borriss (2004) specifically the auxin IAA.

Auxin is a phytohormon that controls and modulates many aspects of plant growth and development, plays a central role in cell elongation and roots initiation (Taiz & Zeiger, 2013). In this study FZB42 was capable of producing indole compounds in suplemented and no-suplemented L-tryptophan (Trp) medium. Without Trp the bacteria production of indole compounds was five times lesser. Idriss et al., (2007) using FZB42 found results indicating that the Trp-dependent synthesis of auxins and plant growth promotion are functionally related.

The morphometric variables showed that the higher concentration of bacteria ($FZB42^{11}$) reduced seedlings growth, with averages lowers than the control, probably related an excess of

metabolites released, as indolic compounds. The IAA secreted by bacteria acts in conjunction with the plant's endogenous IAA. Thus, the impact of bacterial IAA can be positive or negative on plants, depending the produced amount and the sensitivity of the plant tissue to IAA (Ali, Sabri & Hasnain, 2010; Spaepen, Vanderleyden & Remans, 2007), determining whether that bacteria promotes or inhibits plant growth (Duca, Lorv, Patten, Rose & Glick, 2014).

In addition, there is the possibility that bacteria can act on signaling to improve or reduce the plant endogenous auxin synthesis. In the case of molecular signals involved in communication with host plants, the microbial auxin may have a role in interfering with developmental pathways and alter auxin biosynthesis (Contesto et al., 2010).

Some studies address the dose of bacteria acting on the growth of plants for possible action of auxins: (i) seedlings of *V. radiata* treated with PGPR enhanced the endogenous IAA content of roots and leaves. It was observed that IAA produced in roots can be transported to leaves. All bacterial treatments tested recorded reduction in root length that might be due to the production of high levels of IAA inducing an inhibitory effect on root length. Growth promotion reflected the stimulatory effect of low levels of bacterial IAA (Ali et al., 2010); and (ii) bacterial culture filtrate of *Bacillus amyloliquefaciens* FZB42 (same strain used in the present study) productor of IAA applied to duckweed (*Lemna minor*) plants increased fresh weight compared with the control plants. Plant growth promotion was even more pronounced when bacterial cells were applied at the appropriate concentration. Exceeding this concentration of cells, resulted in a significant reduction of plant fresh weight (Idris et al., 2007).

Roots may require minimal concentrations of auxin to grow, and the growth is strongly inhibited by concentrations that promote the elongation of stems and coleoptile (Taiz & Zeiger 2013). The plant root is more sensitive to variability in IAA concentrations, and its response ranges from elongation of the primary root, formation of lateral and adventitious roots, to inhibition of growth (Davies 1995). In dicots, IAA induces lateral-roots formation (McSteen, 2010). In this way, is highlighted the higher number of fine root and lower volume of roots of 'SC' inoculated with FZB42[11], suggesting that these concentration over releasing IAA. Endogenous overproduction of auxin results in an increase in the number of lateral roots (King, Stimart, Fisher & Bleecker, 1995). López-Bucio et al. (2007) found in parallel or as a consequence of primary root–growth inhibition, many lateral roots emerge in plants of *Arabidopsis* WT inoculated with *B. megaterium*, suggestin a effects mediated by auxin.

An example of IAA conjugation can be observed on tobacco (*Nicotiana tabacum*) plants that has an IAA regulatory system that maintains IAA at nontoxic physiologically appropriate

levels. One way to inactivated is converting free IAA to conjugated forms to sugars, amino acids, or peptides (Sitbon et al., 1992).

Zhang et al. (2008) reported that the total chlorophyll concentration is more abundant (88% increased) 2 weeks after exposure to *Bacillus subtilis* GB03, compared with control in *Arabdopsis thaliana.* It is known that IAA also have a relationship with chlorophyll. IAA can retarded chlorophyll loss, this effect was observed in leaf of romaine lettuce (*Lactuca sativa*) (Aharoni, 1989), and IAA prevented the loss of chlorophylls throughout the aging of chloroplasts in wheat (*Triticum aestivum*) (Misra & Biswal, 1980). In addition, the increments on chlorophyll content in inoculated plants can be directed by action of siderophores produced by FZB42 (Szilagyi-Zecchin et al., 2015a), because it can be providing more iron for plant. Iron is essential for several steps of chlorophyll biosynthesis and stabilization (Marschner, 2011).

In this study, the FZB42 inoculation enhanced the photosynthetic apparatus. At the $FZB42^{9}$ and $FZB42^{10}$, the plants showed increases of the chlorophyll and, as a consequence of photosynthetic activity, it was accompanied by increases in total soluble proteins, soluble sugars and also the dry matter was increased on shoots and roots, and the area of aerial part of the seedlings of the two tomato cultivars, especially at $FZB42^{10}$.

As already noted, the concentration of $FZB42^{11}$ caused alterations at different form that was observed for the lowest concentrations, with morphological changes, decreasing roots and shoots. And this doses interfered in physiological aspects, in which the plants presented less sugars and proteins despite to has more chlorophyll. These differences may be due to a cascade of reactions mainly driven by auxinic action (Talboys, Owen, Healey, Withers & Jones, 2014). Not losing sight that caused changes are always linked to an amount of auxin imposed according to the bacteria doses applied.

Some aspects of photosynthesis, as the chlorophylls that capture light energy, soluble proteins (about half is Rubisco) which reduce the CO_2 and soluble sugars which are the final product (Taiz and Zeiger, 2013), were measured in this study. In addition, morphometric variables of the plant were accessed and increases in plant growth were obtained at an appropriated FZB42 concentration. By the consequence, a larger shoot allows a better photosynthetic rate, which implies in more soluble sugars to the growth or reserve for the following seedlings stages, contributing to facilitate a better establishment of plants on the field.

5.5 Conclusions

The use of FZB42 bacteria suspension in the inoculation solution, equivalent to the concentration of $6x10^{10}$ CFU mL^{-1}, improved, in both tomato cultivars, the roots and shoots growth, as well as, improved, total soluble sugars and total soluble protein on leaves. The plant growth promoting or growth reducing effects are closely related to the capacity of FZB42 to release of indolic compounds.

Acknowledgement

Our thanks go to Omex® Agrifluids of Brasil Ltda, for providing the strain of bacteria. This work was supported by Coordination for the Improvement of Higher Education Personnel (CAPES), and by the National Counsel of Technological and Scientific Development (CNPq) [308029/2015-1 –PQ2]

5.6 References

Aharoni, N. (1989). Interrelationship between ethylene and growth regulators in the senescence of lettuce leaf discs. *Journal of Plant Growth Regulation*, *8*, 309-318.

Ali, B., Sabri, A. N., & Hasnain, S. (2010). Rhizobacterial potential to alter auxin content and growth of *Vigna radiata* (L.). *World Journal of Microbiology and Biotechnology*, *26*(8), 1379-1384.

Arkhipova, T. N., Veselov, S. U., Melentiev, A. I., Martynenko, E. V., & Kudoyarova, G. R. (2005). Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. *Plant Soil*, *272*, 201–209.

Asghar, H. N., Zahir, Z. A., Arshad, M., & Khaliq, A. (2002). Relationship *in vitro* production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils*, *35*, 231-237.

Asghar, H. N., Zahir, Z. A., & Arshad, M. (2004). Screening rhizobacteria for improving the growth, yield and oil content of canola (*Brassica napus* L.). *Australian Journal of Agricultural Research*, *55*, 187–194.

Chauhan, H., Bagyaraj, D. J., & Sharma, A. (2013). Plant growth-promoting bacterial endophytes from sugarcane and their potential in promoting growth of the host under field conditions. *Experimental Agriculture*, *49*(01), 43-52.

Chen, X. H., Koumoutsi, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I., Morgenstern, B., Voss, B., Hess, W.R., Reva, O., Junge, H., Voigt, B., Jungblut, P.R., Vater, J., Süssmuth, R., Liesegang, H., Strittmatter, A., Gottschalk, G. & Borriss, R. (2007). Comparative analysis of the complete genome sequence of the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nature Biotechnology, 25*, 1007-1014.

Contesto, C., Milesi, S., Mantelin, S., Zancarini, A., Desbrosses, G., Varoquaux, F., ... & Touraine, B. (2010). The auxin-signaling pathway is required for the lateral root response of *Arabidopsis* to the rhizobacterium *Phyllobacterium brassicacearum*. *Planta*, *232*(6), 1455-1470.

Du, C. X., Fan, H. F., Guo, S. R., & Tezuka, T. (2010). Applying spermidine for differential responses of antioxidant enzymes in cucumber subjected to short-term salinity. *Journal of the American Society for Horticultural Science, 135*(1), 18-24.

Duca, D., Lorv, J., Patten, C. L., Rose, D., & Glick, B. R. (2014). Indole-3-acetic acid in plant–microbe interactions. *Antonie Van Leeuwenhoek, 106*(1), 85-125.

Gordon, S.A. & Weber, R.P. (1951).Colorimetric estimulation of indole acetic acid. *Plant Physiology, 26*, 192-195.

Idris, E. E. S., Bochow, H., Ross, H. & Borriss, R. (2004). Use of *Bacillus subtilis* as biocontrol agent. VI. Phytohormone like action of culture filtrates prepared from plant growth-promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. *Journal of Plant Disease and Plant Protection, 111*, 583-597.

Idris, E. E., Iglesias, D. J., Talon, M., & Borriss, R. (2007). Tryptophan dependente production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Molecular Plant-Microbe Interactions, 20,* 619-626.

King, J. J., Stimart, D. P., Fisher, R. H., & Bleecker, A. B. (1995). A mutation altering auxin homeostasis and plant morphology in *Arabidopsis*. *Plant and Cell, 7*, 2023–2037.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*, 350-382.

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Current Protocols in Food Analytical Chemistry, F4.3*, 1-8.

López-Bucio, J., Campos-Cuevas, J. C., Hernández-Calderón, E., Velásquez-Becerra, C., Farías-Rodríguez, R., Macías-Rodríguez, L. I., & Valencia-Cantero, E. (2007). *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions, 20*(2), 207-217.

Lynch, J. P., & Ho, M. D. (2005). Rhizoeconomics: Carbon costs of phosphorus acquisition. *Plant and Soil, 269*, 45-56.

Maldonade, I. R., Carvaho, P. G. B., & Ferreira, N. A. (2013). *Protocolo para a Determinação de Açucares Totais em Hortaliças pelo Método de DNS*. (Comunicado Técnico nº 85). Brasília: Embrapa Hortaliças.

Marschner, H. (2011). *Marschner's mineral nutrition of higher plants*. 3th ed. Academic press Elsevier

Misra, A. N. & Biswal, U. C. (1980). Effect of phytohormones on the chlorophyll degradation during aging of chloroplasts *in vivo* and *in vitro. Protoplasma, 105,* 1-8.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagente for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428.

Ongena, M., & Jacques, P. (2008). *Bacillus lipopeptides*: versatile weapons for plant disease biocontrol. *Trends in Microbiology, 16,* 115-125.

Rodrigues Neto, J., Malavolta Jr, V. A., & Victor, O. (1986). Meio simples para isolamento e cultivo de *Xanthomonas campestris* pv. *citri* tipo B. *Summa Phytopathologica, 12,* 16.

Silva, F. A. S., & Azevedo, C. A. V. (2002). Versão do programa computacional Assistat para o sistema operacional Windows. *Revista Brasileira de Produtos Agroindustriais, 4*(1), 71-78.

Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiology Reviews, 31*, 425-448.

Sitbon, F., Hennion, S., Sundberg, B., Anthony Little, C. H., Olsson, O., & Sandberg, G. (1992). Transgenic tobacco plants coexpressing the *Agrobacterium tumefaciens iaaM* and *iaaH* genes display altered growth and indoleacetic acid metabolism. *Plant Physiology, 99,* 1062-1069.

Szilagyi-Zecchin, V. J., Mógor, Á. F., Ruaro, L., & Röder, C. (2015a). Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. *Plantarum* FZB42 em sistema de cultivo orgânico. *Revista de Ciências Agrárias 38,* 26-33.

Szilagyi-Zecchin, V. J., Klosowski, A. C., Ikeda, A. C., Hungria, M., Galli-Terasawa, L. V., Kava-Cordeiro, V., Glienke, C., Mógor, À. F. (2015b). Potential inoculant strains of Brazilian endophytic bacteria for maize (*Zea mays* L.) growth promotion. *International Journal of Agronomy and Agricultural Research, 7*,(4), 128-134.

Taiz, L., & Zeiger, E. (Eds). (2013). *Plant physiology*. Sunderland: Sinauer Associates, Inc. Publishers.

Talboys, P. J., Owen, D. W., Healey, J. R., Withers, P. J., & Jones, D. L. (2014). Auxin secretion by *Bacillus amyloliquefaciens* FZB42 both stimulates root exudation and limits phosphorus uptake in *Triticum aestivum*. *BMC Plant Biology, 14*(1), 51.

Thomas, P., & Upreti, R. (2015). Evaluation of tomato seedling root-associated bacterial endophytes towards organic seedling production. *Organic Agriculture,* 1-10, doi:10.1007/s13165-015-0111-9

Vardharajula, S., Zulfikar Ali, S., Grover, M., Reddy, G., & Bandi, V. (2011). Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *Journal of Plant Interactions*, *6*(1), 1-14.

Zhang, H., Xie, X., Kim, M. S., Kornyeyev, D. A., Holaday, S., & Paré, P. W. (2008). Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. *The Plant Journal*, *56*(2), 264-273.

Table 1 –ANOVA analyses of morphometrics and biochemical variables. (*) $p \leq 0.05$ and (**) $p \leq 0.01$.

	Morphometrics variables				*Biochemical variables ($\mu g\ g^{-1}$)*					
	Root volume (cm^3)	**Root dry weight (g)**	**Shoot area (cm^2)**	**Shoot dry weight (g)**	**Chlorophyll *a***	**Chlorophyll *b***	**Total chlorophyll**	**Carotenoids**	**Total soluble sugars**	**Total soluble proteins**
Tomato cultivar (TC)	**	**	**	**	**	**	**	**	**	**
Bacteria doses (BD)	**	**	**	**	*	*	*	*	**	**
TC x BD	**	**	**	**	*	*	*	*	**	**

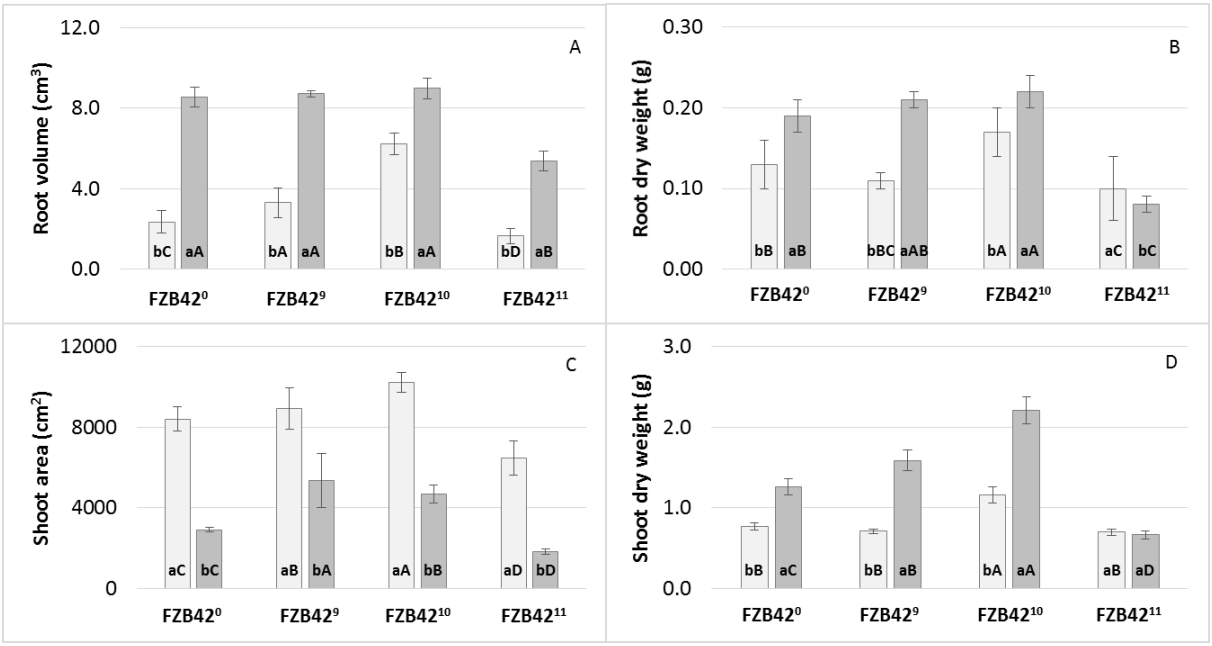

Figure 1. Morphometrics data of tomato seedlings by inoculation with three bacterial concentrations of *Bacillus amyloliquefaciens* FZB42, and a control, at 35 days after planting. Mean and standard deviation were represents in light bar for 'Santa Cruz Kada Gigante' and dark bar for 'Serato'. Equal letters, lowercase between cultivars and uppercase between concentrations of the same cultivar do not differ by Tukey test, $p \leq 0.01$.

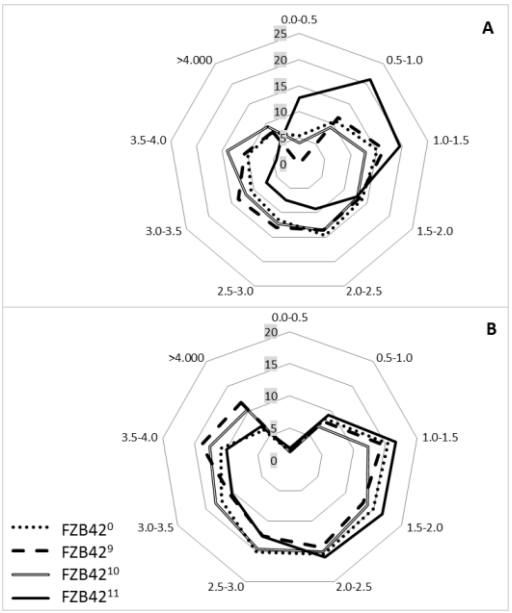

Figure 2. Thickness of tomato seedlings roots, by inoculation with three bacterial concentrations of *Bacillus amyloliquefaciens* FZB42, and a control, at 35 days after planting , fractionated in intervals of 0.5 mm, shown in percentage values of each treatment. (A) 'Santa Cruz Kada Gigante' (B) 'Serato'.

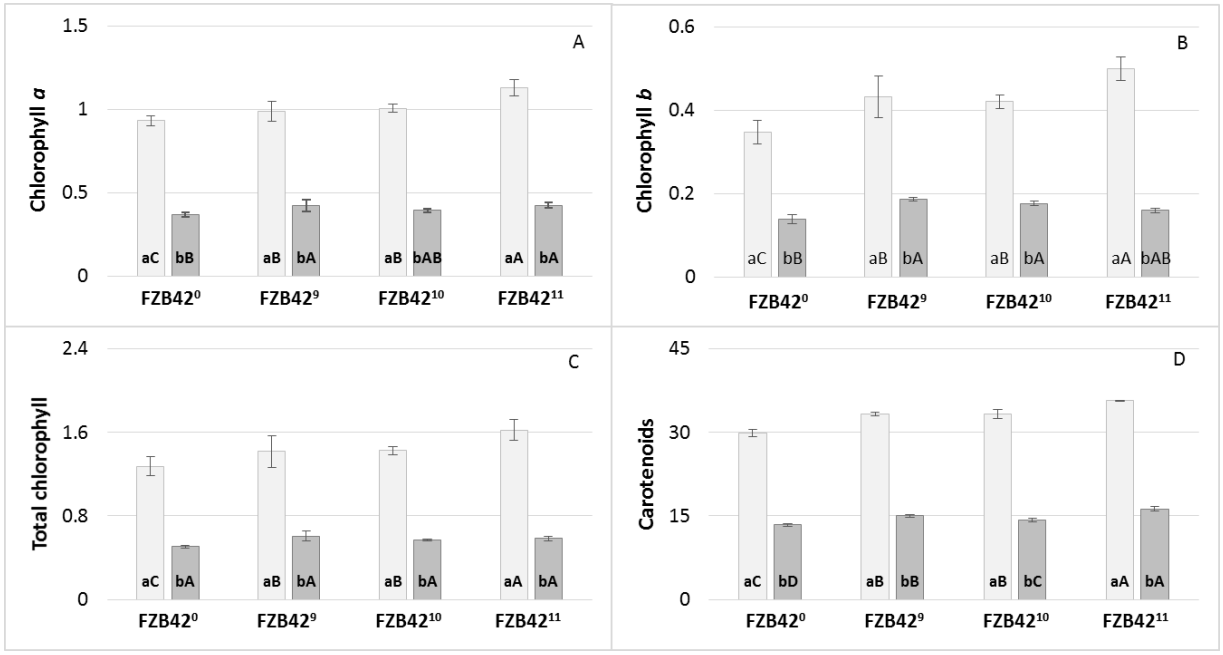

Figure 3. Pigments (µg g^{-1}) of tomato seedlings leaves, by inoculation with three bacterial concentrations of *Bacillus amyloliquefaciens* FZB42, and a control, at 35 days after planting. Mean and standard deviation were represents in light bar for 'Santa Cruz Kada Gigante' and dark bar for 'Serato'. Equal letters, lowercase between cultivars and uppercase between concentrations of the same cultivar do not differ by Tukey test, $p \leq 0.05$.

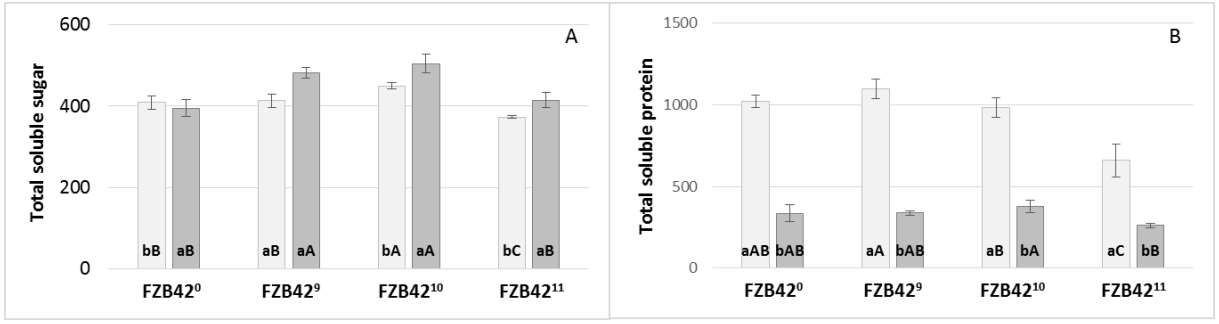

Figure 4. (A) Total soluble sugars (µg g^{-1}) and (B) total soluble proteins (µg g^{-1}) of tomato seedlings leaves, by inoculation with three bacterial concentrations of *Bacillus amyloliquefaciens* FZB42, and a control, at 35 days after planting.Mean and standard deviation were represents in light bar for 'Santa Cruz Kada Gigante' and dark bar for 'Serato'. Equal letters, lowercase between cultivars and uppercase between concentrations of the same cultivar do not differ by Scott-Knott test, $p \leq 0.01$.

6 CAPÍTULO IV – YIELD AND METABOLIC CHANGES ON TOMATO INOCULATED WITH *Bacillus amyloliquefaciens* FZB42

Abstract
The technology for crop production faced fast-growing food and energy demands, but driven by a new approach, the answer for those demands must be socially and environmentally conscious. In organic systems, the options for plant growth promotion are rather limited as the common growth-enhancing chemicals are unacceptable. In this respect, use of bacteria that promote the plant growth offer several benefits and play a main role in sustainable agriculture. The plant growth promoting bacteria *Bacillus amyloliquefaciens* FZB42 was tested in two doses (BAP-1, $1.5x10^9$ CFU mL^{-1}; and BAP-2, $6x10^{10}$ CFU mL^{-1}) by morphometric, physiological and nutritional analyses, aiming to identify their potential as inoculant or bio-fertilizer on organically grown tomato. Both doses improved significantly the yield due to the higher number of fruits, around four, and enlargement of fruit diameter. This led to increments of about 1 kg per plant, and considering the extrapolation of this yield for one hectare (12,820 plants ha^{-1}), the results shows increments about 11.76, and 13.23 ton ha^{-1} at the doses BAP-1 and BAP-2, respectively. The leaves of inoculated plants had higher amounts of soluble sugars and total free amino acids and the fruits presented highest content of total soluble protein and total free amino acids. The inoculation with FZB42 increased on N, Fe and Mn leaf content. These results indicate that the inoculation with this strain is a promising inoculant or bio-fertilizer for tomato that can contribute to the sustainability of tomato production.

Keywords: plant growth promoting bacteria, nutrient analyses, bio-fertilizer, metabolites, organic production, inoculation

6.1 Introduction

According to FAO, the world production of tomatoes (*Solanum lycopersicum* L.) in 2013 was of 163.9 million tons. Brazilian annual production is around 4.1 million tons, the 8 th largest worldwide. The Brazilian area with tomato cultivation at 2016 season is estimated of 59.069 ha, with production foreseen around 3.62 million tons (IBGE, 2016).

Bacillus amyloliquefaciens $FZB42^T$ [Krebs et al. 1998] the type strain for *B. amyloliquefaciens* subsp. *plantarum* (Borris et al. 2011), has the whole genome sequence determined in 2007 (Chen et al. 2007), as the first representative of gram-positive plant growth-promoting bacteria. *B. amyloliquefaciens* strains belonging to subsp. *plantarum* and are distinguished from other representatives of endospore-forming *B. amyloliquefaciens* by their ability to colonize plant rhizosphere, to stimulate plant growth, and to suppress competing phytopathogenic bacteria and fungi. Due to their biofertilizer and biocontrol properties, they

are becoming increasingly important as a natural alternative to agrochemical (Qiao et al. 2014). This ability to colonize surfaces of plant roots is a prerequisite for phytostimulation.

The rhizosphere competence is linked to the capability to form sessile, multicellular communities (Borris 2013). *Bacillus* has an additional beneficial ability to form heat- and desiccation-resistant spores (Emmert & Handelsman 1999) making high feasibility to be formulated for commercially available (Ongena & Jacques 2008).

Bacillus are bacteria that have a positive influence on plant growth, stimulating seed germination (Szilagyi-Zecchin et al. 2014), shoot development (Szilagyi-Zecchin et al. 2015b), root-hair formation and elongation (Rahman et al. 2002). Including increases the productivity of commercial interest plants like tomato, pepper (García et al. 2004), and onion (Harthmann et al., 2009; Harthmann et al., 2010).

Fruit set and fruit growth and development depends the uploads of metabolites, mineral elements, and water from source organs. Most of that are transported from photosynthetic leaves (Lytovchenko et al. 2011). The study of source-sink relation, like transport and partitioning into competing organs, is therefore of special interest on improvement of fruit yield and quality (Ho 1996). In this context the plant growth promoting bacteria can causes modulation on primary metabolism in plants, changing the production of carbohydrates, proteins and amino acids (Canellas et al. 2012; Kang et al. 2014; Vardharajula et al. 2014).

The plant production technology faced fast-growing food and energy demands, but driven by a new approach, the answer for those demands must be socially and environmentally conscious (Szilagyi-Zecchin et al. 2016). In organic systems, the options for plant growth promotion are rather limited as the common growth-enhancing chemicals such as fertilizers and pesticides are unacceptable. In this respect, use of microorganisms that promote the plant growth offer several benefits (Thomas & Upreti 2015), and play a main role in sustainable agriculture (Jha et al. 2013).

The use of plant growth promoting bacteria as crop inoculants for biofertilization, and phytostimulation, would be an attractive option to reduce the use of chemical fertilizers, which also cause environmental pollution (Bloemberg & Lugtenberg 2001). In this context, search for bacteria that contribute to the yield increasing gain greater significance, above all at organic production. For those reasons, the aim of this work was to assess the yield, physiological and nutricional alterations on organically grown tomato plants, inoculated with *Bacillus amyloliquefaciens* subs. *plantarum* FZB42 bacteria, looking for its potential as inoculant or bio-fertilizer.

6.2 Materials and methods

Seeds of the tomato 'Serato F1' (TopSeed®) were used, a cultivar with undetermined growth rate. The inoculation was done using *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42^{T} (Omex® Agrifluids do Brasil Ltda), Gram positive, isolated from soil (Krebs et al, 1998) and stocked as 10A6 strain in the *Bacillus* Genetic Stock Center (BGSC, Ohio, U.S.A.).

6.2.1 Inoculation

A bacterial cell suspension was incubated at 30°C for 24 hours at 150 rpm in Luria-Bertani (10 g tryptone, 10 g NaCl, and 5 g yeast extract per 1 L) broth. Then the suspension was centrifuged at 10.000 x *g* and re-suspended in saline solution (0.85% NaCl). The bacteria suspension was adjusted to compose the treatments inoculum: (Control) only distilled water (BAP-0); $1.5x10^{9}$ CFU mL^{-1} mL^{-1} (BAP-1); $6x10^{10}$ CFU mL^{-1} mL^{-1} (BAP-2). Seeds were inoculated in the proportion of 320 uL g^{-1} of seed. After inoculation, the seeds were left drying in the shade, on paper towels, and then immediately sown.

6.2.2 Field assay

Seeds were sown in expanded polystyrene trays, with 200 cells filled with commercial substrate (Bioplant®) and placed at polyethylene film covered nursery. At 25 days after germination, seedlings were planted at a polyethylene tunnel type greenhouse (15 x 25 m) with drip irrigation at the Organic Production Research Area, were organic production system was implemented 10 years ago, at the Federal University of Paraná (UFPR), Curitiba city, Paraná State, South of Brazil (25º25'S, 49º 06'W; 920 m of altitude). The climate according to Koppen classification is temperate, humid mesothermal (Cfb), with annual precipitation between 1,400 and 1,800 mm, and well distributed rainfall. The soil nutrient analysis showed the following values: pH ($CaCl_2$) = 6.5; pH SMP = 6.6; Al^{+3} = 0; H + Al = 2.2 cmolc dm^{-3}; Ca^{+2} = 8.0 cmolc dm^{-3}; Mg^{+2} = 4.1 cmolc dm^{-3}; K^{+2} = 1.45 cmolc dm^{-3}; P = 115.00 mg dm^{-3}; C = 38 g dm^{-3}; V% =86; CTC = 15.75 cmolc dm^{-3}.

Before the planting, according to Brazilian organic production regulation, it was used the amount equivalent to 5 ton ha^{-1} of the organic compost showing the following nutrient values: pH 7.1; P= 14.00 g Kg^{-1}; K= 11.3 g Kg^{-1}; Ca= 31.7 g Kg^{-1}; Mg= 6.8 g Kg^{-1}; with C:N ratio = 27.6. The plants were established with 55 cm between them and 1.20 m between lines, resulting in a population of 12,820 plants per hectare, which were tying vertically using plastic tapes, keeping two stems per plant.

The yield was assessed throughout the cycle by weekly harvest of the red-ripe fruit. To standardize, the color adopted at harvest was mature red, which is characterized as more than 90% of the fruits surface are red (MAPA 2002). All fruits of nine rachis per plant, were weighed and transverse diameter were measured. The design was completely randomized with three treatments and seven replications, each replication consisted of four useful plants, thus with 84 plants altogether

6.2.3 Biochemical analysis in leaves and fruits

Four leaf per repetition, at 135 days after sowing (DAS), were collected from the middle third of the plant between 9:00 am and 10:00 a.m.. The leaflets at the middle parte of the leaf were macerated with liquid nitrogen in a mortar, until obtaining a fine powder. Five tomato fruits on red-ripe stage per repetition were collected from the middle third of the plants, also at 135 DAS. The skin and seeds were removed and the pericarp was used. The samples were blended or homogenized on highest speed for 20 s. Values were expressed in mg of metabolite per g of fresh weight of leaf and fruit after spectrophotometric analysis. The samples were performed in triplicates.

Sugars – The sugar extraction was performed with a 0.3 g of fresh matter on 2 mL of distilled water, and agitated by vortexing for 20 s. After centrifugued at 9335 x *g* for 15 min the sample supernatant was collected for hydrolysis and subsequent determination of total soluble sugar and for determination of reducing sugar adapted from Maldonade et al. (2013). For hydrolysis was done in 1 mL of sample was added 1 mL of HCl 2 N, it was boilied for 10 min, then cooled in ice bath for 5 min, after that bufferd with 1 mL of NaOH 2N (Maldonade et al. 2013). This final solution were used to establish the total soluble sugar. The both analyses (reducing and total sugars) were determined with colorimetric reaction with 3.5-Dinitrosalicylic acid (DNS) (Miller 1959). A samples aliquot of 1 mL was mixed with 1 mL of the DNS reagent and incubated in a boiling water bath for 15 min. After incubation added 1 mL

of Rochelle salt (tartrate of sodium and potassium 40% w v^{-1}) and than cooling in ice bath for 10 min. The absorbance was measured at 540 nm. The standard curve for reducing and total sugars was obtained with glycose 5.5 mM, with values between 50 and 800 µg mL^{-1} that generated the equation: y = 0.0033 x - 0.101, with an R^2 = 0.9947.

Total soluble proteins – extraction of soluble proteins was performed with 0.5 g of fresh matter in 1.5 mL of buffer, according to Du et al. (2010) with modification: phosphate buffer pH 7.5 and 100 mM, with the addiction of 1 mM EDTA, 3 mM 1,4-dithiothreitol (DTT), 4% polyvinylpyrrolidone (PVP) (w v^{-1}) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The solution were homogeneized by vortexing for 10 s at low speed, and after centrifugued at 9,000 x *g* for 15 min. The supernatant was collected for measuring at 595 nm by the protein dye-binding method of Bradford (1976). The standard curve was built with bovine serum albumin (BSA) at 0.2% (w v^{-1}), with values between 28 and 140 µg mL^{-1} that generated the equation: y = 0.0281 x + 0.0153, with an R^2 = 0.9954.

Total free amino acids - amino acids were extracted by Withers et al. (2002) method, with adaptations: 0.3 g of fresh matter and 4 mL of distilled water were heated in a boiling water bath for 25 min, immediately afterwards, mixed thoroughly for 2 s in vortex, and allowed to cool at 20 °C. A supernatant of 1.5 ml was removed and centrifuged at 9,000 x *g* for 10 min. Colorimetric reaction was performed according Magné and Larher (1992), 1 mL of sample was added to 0.5 mL of citrate buffer 0.2 M pH 4.6, subsequently 1 ml of ninhydrin solution were added to the reaction mixture. Thereafter the samples were brought to boiling bath for 15 min, then were cooled to room temperature and 3 ml of 60% ethylic alcohol in water was added. Absorbance of the chromophore was read at 570 nm. The standard curve was performed with asparagine and glutamine 2 mM in a range of 28-140 mg mL^{-1}, that generated the equation: y = 0.01 x + 0.0741, with an R^2 = 0.9908.

6.2.4 Nutrient content of leaves

Two fully expanded leaves per plant, at 135 DAS, were collected from the middle third of the plant. The leafs were washed with detergent and distilled water. The midrib was eliminated and only the leaf blade were used for analysis. The samples were dried at 65 °C until constant weight.

The leaves content of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese and zinc were determined according Bataglia et al. (1983).

6.2.5 Statistical analyses

Normality of data was verified by Kolmogorov-Smirnov test, and homogeneity of variances were submitted to Bartlett test. Next, data were submitted to ANOVA, and means were compared at 5 and 1% of significance by Scott-Knott test. The software Assistat 7.7 Beta (Silva & Azevedo 2002) was used for statistical analyses.

6.3 Results

The yield obtained by inoculation was significantly higher for both doses tested, with no differences between them. This led to increments of about 1 kg per plant (917.64 and 1032.19 g per plant) (Figure 1B). This improvement of yield reflected the higher number of fruits per plant in inoculated plants, around 4 (Figure 1C). Considering the extrapolation of this yield for one hectare, the results shows increments about 11.76, and 13.23 ton ha^{-1} respectively according to the increasing of doses (Figure 1D).

At the classification of fruits, according to size, can be seen that there was a significant difference in the smaller size class (50-65 mm), in which BAP-1 and BAP-2 showed fewer fruits compared to control (BAP-0) (Table 1). While in the class of 80-100 mm, the most valuable at the market, all treatments with inoculation showed higher fruit numbers.

The inoculation increased the fruis number and their size. Hence some changes in metabolism that can justify these effect were also observed The leaf of inoculated plants, in both doses, had higher amounts of total soluble sugars and BAP-1 showed also more reducing sugar (Table 2). A pattern of increases on metabolites of leaf was observed in total free amino acid content, in treatments at BAP-1 and BAP-2. And the total soluble proteins did not differ from control. In fruits, the biochemical analysis reveled a raising in total soluble protein and in total free amino acids.

Plants inoculated with the FZB42 in all doses tested raised content of nitrogen (N), until appropriate levels for the present development stage, during fruiting (Table 5).

The contents of the phosphorus (P), potassium (K), magnesium (Mg), boron (B), calcium (Ca) and sulfur (S) in inoculated plants had no significant differences.

Plants inoculated with BAP-2 had zinc (Zn) increase and copper (Cu) level disminiched. While plants submited to BAP-1 and BAP-2 showed more iron (Fe) and manganese (Mn) (Table 3).

6.4 Discussion

The plants inoculated with *B. amyloliquefaciens* FZB42 in both doses tested showed highest yield than the control, by increasing in the number of fruits per plant (about four), also by increasing the number of fruits with transverse diameter among 80-100 mm, which may have influenced a slight increase on averages of fruits weight. Corroborating these tomato increase in yield data by bacteria inoculation, were reported that *Bacillus liqueniformis* CECT 5106 improved fruits number and diameter (García et al. 2004), *Bacillus subtilis* BEB-13bs increased the weight, lenght and number of fruits (Mena-Violante et al. 2007), and *Pseudomonas* spp. increased the fruit yield per plant (Gravel et al. 2007).

The improvement on number of fruits per plant found in this work, may be due to the greater number of fertile flowers. There are reports about the key role of auxins in the transition of ovary into tomato fruit, in wich is initiated by successful pollination and fertilization, triggering the fruit-set and initiating fruit development (Serrani et al. 2008; Jong et al. 2009). IAA acts in many physiological processes; affects photosynthesis and pigment formation; mediates responses to florescence; controls biosynthesis of various metabolites; controls processes of vegetative growth; and more specifically acts in cell division and differentiation (Spaepen & Vanderleyden 2011; Taiz & Zeiger 2013). It is known that this strain FZB42 can produce indole acetic acid (IAA) (Szilagyi-Zecchin et al. 2015a; Idris et al. 2007). The IAA produced by bacteria acts together with the endogenous plant supply, and the impact of bacterial IAA are related to plant tissues sensibility (Ali et al. 2010). Beyond that, exist the possibility of bacteria interference in developmental pathways and in alterations on auxin biosynthesis (Contesto et al. 2010) or signalling to activate basipetal auxin transport in the host (Zhang et al. 2007).

The largest caliber of fruit can be connected to the additional effect of bacterial FZB42 auxin, because this hormone stimulates growth by cell expansion and cell division (Taiz & Zeiger 2013). On tomato, exogen auxins (synthetic) improved the fruit size and consequently also the average fruit weight (Tonder & Combrink 2003). More recently, Su et al. (2014)

demonstrated that the Aux/IAA pathway contributes to controlling the pericarp fruit development, especially fruit size and cell size determination in tomato. Beyond that, as sink organs, tomato fruits are dependent on the translocation of sucrose, amino acids, and organic acids to the developing fruit cells. The rate of uptake of these photoassimilates from the leaves is governed by the metabolic activity of the fruit (Ho 1988).

'Serato'-FZB42 interaction, altered the primary metabolism with increases in sugars and amino acids in leaves. Tomato plants cultivated on field and inoculatend with *Paenibacillus polymyxa* and *Bacillus megaterium* var *phosphaticum* also had total sugars raised in leaves. (El-Yazeid & Abou-Aly 2011). In the same way, Kang et al. (2014) verified a similar situation in leaves with highest content of sugars and amino acids, inoculating *Bacillus megaterium* mj1212 in mustard (*Brassica juncea*), and according to the authors, would be a reason for plant growth improvement.

In the present work, the proteins and amino acids were increased in red rip tomato fruits of inoculated plants. Probably by through a chain reaction, happened that the greatest amount of amino acids present in the leaves were redistributed for fruits, due to the higher amount of nitrogen found in inoculated plants leaves. This is justified because the nitrogen (N) taken up by plants is incorporated into C chains to produce amino acids, which are components of proteins stored in plant tissues (source tissues). By the time of fruit filling, the protein reserves are metabolized and broken into small amino acid molecules and N is redistributed to the sink (new tissues) (Marschner 2011).

Tomato plants inoculated with FZB42 altered the nutricional status of N and micro elements in leaves when comparing to the non-inoculated plants. Esitken et al. (2006) reported increases on nutrient content of leaves by inoculation of *Pseudomonas* BA-8 and *Bacillus* OSU-142 affecteing among others the N, Fe, Mn, Zn on sweet cherry (*Prunus avium*).

The N amout detected in plants with FZB42 was incresed with the doses. The nitrogen is one of the most abundant element in the plants. It is a primary constituent of the nucleotides and amino acids that will form the structure of nucleic acids and proteins (Taiz & Zeiger 2013). In soils, the forms of inorganic N is nitrate (NO_3^-) and ammonium (NH_4^+). The ammonium derived from nitrate or directly from ammonium uptake by ammonium transporters is further assimilated into amino acids via the GS/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle (Xu et al. 2012).

The plant growth promoting bacteria can increase the nitrate (NO_3^-) uptake capacity by two types of mechanisms: indirectly, as a consequence of stimulated lateral root development;

and directly, by stimulating NO^{-}_{3} transport systems (Mantelin and Touraine, 2004). The inorganic nitrogen (i.e. NO_3^- and NH_4^+) taken up by roots are incorporated into glutamine and glutamate, which is used to synthesize other amino acids and nitrogenous compounds. This process happens either in root or shoot tissue, depending the molecular species of nitrogen taken up and the carbon/nitrogen balance of the plant (Okumoto & Pilot 2011; Marschner 2011). In this work, is possible that the increasing of N in plants inoculated with FZB42, was not released to increase soluble proteins but it was efficiently used for amino acid synthesis on leaves. Moreover, these amino acids were transportated to the fruit, where we saw that the amount of amino acids and proteins were increased, while the amino acids synthesized are delivered to the so-called sink organs like, roots, young leaves, and fruits (Okumoto & Pilot 2011).

FZB42 was able to enhance in plants iron (Fe) and manganese (Mn) uptake, possibly by the ability to produce siderophores (Szilagyi-Zecchin et al. 2015a). The analysis of the complete genome of the FZB42 verifing that more than 8.5% of the genome is devoted to synthesizing antibiotics and siderophores, the iron-siderophore bacillibactin was detected (Chen et al. 2007). Siderophores are low molecular weight (500–1000 Da) compounds produced by fungi and bacteria, which bind among others Fe^{3+} and Mn^{3+}ions to be transported into the cell, also called chelating agentes (Duckworth et al. 2009; Saraf et al. 2014).

Iron has a biologically importance because is a constituent of cytochrome and others heme or non-heme proteins and a cofactor in many enzymes essential to physiological processes, such as respiration, photosynthesis and nitrogen fixation (Taiz & Zeiger 2013; Marschner 2011). Mn plays an important role in oxidation and reduction processes in plants, such as the electron transport in photosynthesis. Also has a role in chlorophyll production, activation of nitrate-reducing enzyme and enzymes of carbohydrate metabolism. For that roles, the improvement on manganese uptake, would increasing the efficiency of photosynthesis and carbohydrates synthesis (Millaleo et al. 2010; Marschner 2011), contributing to the results obtained in this work, with increases on sugar content of inoculated plants.

Copper and zinc are necessary nutrients in plants. Zinc is a component of enzymes and is involved in the carbohydrates metabolism, and phosphorus compounds (Alloway 2004), and participates in the auxins synthesis (Marschner 2011). Copper is mainly an activator of enzymes involved in metabolism of nitrogen (Marschner 2011). Zinc can reduce the availability of copper due competition for the same sites for absorption into the plant root. Imtiaz et al. (2003) saw that Zn had an adverse effect on the Cu concentration in the plant tissue of wheat (*Triticum aestivum*). On the other hand, maybe Mn had significant synergistic effect on the uptake Zn and

had antagonistic effect on uptake of Cu, what was also reported by Fageria et al. (2002) studing the elements in common bean (*Phaseolus vulgaris*) plants. Those finds could justify the results obtained with inoculation, in which the improvement of Mn and Zn uptake had possible antagonism by reducing Cu amount in tomato leaves.

The inoculation with *Bacillus amyloliquefaciens* FZB42, promoted increased yields in both doses tested by the modulation of metabolism of sugars, proteins and amino acids, and by improve the sink-source relation to the fruits, and also by improve the N, Fe and Mn uptake. This results indicate that the inoculation with this strain is a promising inoculant or bio-fertilizer for tomato that contribute to the sustainability of tomato production.

Acknowledgement

Our thanks go to Omex® Agrifluids of Brasil Ltda, for providing the strain of bacteria. This work was supported by Coordination for the Improvement of Higher Education Personnel (CAPES), and by the National Counsel of Technological and Scientific Development (CNPq), process: 308029/2015-1 –PQ2.

6.5 References

Ali B, Sabri AN, Hasnain S. 2010. Rhizobacterial potential to alter auxin content and growth of *Vigna radiata* (L.). World J Microbiol Biotechnol. 26:1379-1384.

Alloway BJ. 2004. Zinc in soils and crop nutrition. Brussels: International Zinc Association.

Bataglia OC, Furlani AMC, Teixeira JPF, Furlani PR, Gallo JR. 1983. Métodos de análise de plantas, Boletim Técnico 78. Campinas: Instituto Agronômico.

Bloemberg GV, Lugtenberg BJJ. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol. 4:343-350.

Borriss R, Chen XH, Rueckert C, Blom J, Becker A, Baumgarth B, Fan B, Pukall R, Schumann P, Spröer C, Junge H, Vater J, Pühler A, Klenk HP. 2011. Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM7T and FZB42T: a proposal for *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* subsp. nov. and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on complete genome sequence comparisons. Int J Syst Evol Microbiol. 61:1786-1801.

Borriss R. 2013. Molecular Microbial Ecology of the Rhizosphere. Hoboken: John Wiley & Sons. Chapter 83, Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42; p. 883-898.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72(1): 248-254.

Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I, Morgenstern B, Voss B, Hess WR, Reva O, Junge H, Voigt B, Jungblut PR, Vater J, Süssmuth R, Liesegang H, Strittmatter A, Gottschalk G, Borriss R. 2007. Comparative analysis of the complete genome sequence of the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nat. Biotechnol. 25:1007-1014.

Canellas LP, Balmori DM, Médici LO, Aguiar NO, Campostrini E, Rosa RC, Façanha AR, Olivares FL. 2013. A combination of humic substances and *Herbaspirillum seropedicae* inoculation enhances the growth of maize (*Zea mays* L.). Plant soil. 366(1-2):119-132.

Contesto C, Milesi S, Mantelin S, Zancarini A, Desbrosses G, Varoquaux F, Bellini C, Kowalczyk M, Touraine B. 2010. The auxin-signaling pathway is required for the lateral root response of *Arabidopsis* to the rhizobacterium *Phyllobacterium brassicacearum*. Planta. 232(6):1455-1470.

Du CX, Fan HF, Guo SR, Tezuka T. 2010. Applying spermidine for differential responses of antioxidant enzymes in cucumber subjected to short-term salinity. J Am Soc Hortic Sci. 135(1):18-24.

Duckworth OW, Bargar JR, Sposito G. 2009. Coupled biogeochemical cycling of iron and manganese as mediated by microbial siderophores. Biometals, 22(4):605-613.

EI-Yazeid AA, Abou-Aly HE. 2011. Enhancing growth, productivity and quality of tomato plants using phosphate solubilizing microorganisms. Aust J Basic Appl Sci. 7: 371-379.

Emmert EAB, Handelsman J. 1999. Biocontrol of plant disease: A Gram-positive perspective. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 171:1-9.

Esitken A, Pirlak L, Turan M, Sahin F. 2006. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. Sci Hort. 110(4):324-327.

Fageria NK. 2002. Influence of micronutrients on dry matter yield and interaction with other nutrients in annual crops. PAT. 37(12):1765-1772.

FAOSTAT: Food and Agriculture Organization of United Nations [Internet]. 2013. Food and Agricultural commodities production. Available from: http://www.faostat.fao.org.

García JAL, Probanza A, Ramos B, Palomino M, Maņero FJG. 2004. Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. Agronomie. 24(4):169-176.

Gravel V, Antoun H, Tweddell RJ. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem. (8):1968-1977.

Harthmann OEL, Mógor AF, Wordell JAF, Luz WC. 2010 Rizobactérias no crescimento e na produtividade da cebola. Cienc Rural. 40(2):462-465.

Harthmann OEL, Mógor AF, Wordell JAF, Luz WC, Biasi LA. 2009. Tratamento de sementes com rizobactérias na produção de cebola. Cienc Rural. 39(9):2533-2538.

Ho LC. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annu Rev Plant Physiol Plant Mol Biol. 39(1):355-378.

Ho LC. 1996. The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. J Exp Bot. 47:1239-1243.

Hochmuth GJ [Internet]. 2015. Fertilizer management for greenhouse vegetables -Florida greenhouse vegetable production handbook, vol 3, HS787 series, Horticultural Sciences Department, UF/IFAS Extension. Available from: http:// http://edis.ifas.ufl.edu./cv265.

IBGE: Instituto Brasileiro de Geografia e Estatística [Internet]. 2016. Dados de Previsão de Safra. Available from: http://www.sidra.ibge.gov.br/bda/prevsaf/default.asp?t=1&z=t&o=26&u1=1&u2=1&u3=1&u4=1.

Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriss R. 2002. Extracellular phytase activity of Bacillus amyloliquefaciens FZB45 contributes to its plant-growth-promoting effecta. Microbiology. 148(7):2097-2109.

Idris EE, Iglesias DJ, Talon M, Borriss R (2007) Tryptophan dependente production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol Plant-Microbe Interact. 20:619-626.

Imtiaz M, Alloway BJ, Shah KH, Siddiqui SH, Memon MY, Aslam M, Khan P. 2003. Zinc Nutrition of Wheat: II: Interaction of Zinc with other Trace Elements. Asian J Plant Sci. 2:156-160.

Jha PN, Gupta G, Jha P, Mehrotra R. 2013. Association of rhizospheric/endophytic bacteriawith plants: a potential gateway to sustainable agriculture. Greener J Agric Sci. 3:73-84.

Jong M, Wolters-Arts M, Feron R, Mariani C, Vriezen WH. 2009. The *Solanum lycopersicum* auxin response factor 7 (*SlARF7*) regulates auxin signaling during tomato fruit set and development. Plant J. 57:160-70.

Kang SM, Radhakrishnan R, You YH, Joo GJ, Lee IJ, Lee KE, Kim JH. 2014. Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. Indian J Microbiol. 54(4):427-433.

Krebs B, Höding B, Kübart SM, Workie A, Junge H, Schmiedeknecht G, Grosch R, Bochow H, Hevesi M. 1998. Use of *Bacillus subtlis* as biocontrol agent activities and characterization of *Bacillus subtilis* strains. J Plant Dis Protect. 105:181-197.

Lytovchenko A, Eickmeier I, Pons C, Osorio S, Szecowka M, Lehmberg K, Arrivault S, Tohge T, Pineda B, Anton MT, Hedtke B, Lu Y, Fisahn J, Bock R, Stitt M, Grimm B, Granell A, Fernie AR. 2011. Tomato fruit photosynthesis is seemingly unimportant in primary metabolism and ripening but plays a considerable role in seed development. Plant Physiol. 157:1650-1663.

Magné C, Larher F. 1992. High sugar content interferes with colorimetric determination of amino acids and free proline. Anal Biochem. 200:115-118.

Maldonade IR, Carvaho PGB, Ferreira NA. 2013. Protocolo para a determinação de açucares totais em hortaliças pelo método de DNS, Comunicado Técnico 85. Brasília: Embrapa.

Mantelin S, Touraine B. 2004. Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot. 55(394):27-34.

MAPA: Ministério da Agricultura, Pecuária e Abastecimento [Internet]. 2002. Secretaria De Apoio Rural E Cooperativismo, Portaria Nº 85, de 6 de Março de 2002. Available from: http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=consultarLegislacaoFederal.

Marschner H. 2011. Marschner's mineral nutrition of higher plants. 3th ed. Academic press Elsevier

Mena-Violante HG, Olalde-Portugal V. 2007. Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. Sci Hort. 113:103-106.

Millaleo R, Reyes DM, Ivanov AG, Mora ML, lberdi MA. 2010. Manganese as essential and toxic element for plants transport, accumulation and resistance mechanisms. J Soil Sci Plant Nutr. 10(4):470-481.

Miller GL. 1959. Use of dinitrosalicylic acid reagente for determination of reducing sugar. Anal Chem, 31(3):426-428.

Okumoto S, Pilot G. 2011. Amino acid export in plants: a missing link in nitrogen cycling. Mol plant. 4(3):453-463.

Ongena M, Jacques P. 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 16:115–125.

Qiao JQ, Wu HJ, Huo R, Gao XW, Borriss R. 2014. Stimulation of plant growth and biocontrol by *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 engineered for improved action. Chem Biol Technol Agric. 1(1):1-14.

Rahman A, Hosokawa S, Oono Y, Amakawa T, Goto N, Tsurumi S. 2002. Auxin and ethylene response interactions during *Arabidopsis* root hair development dissected by auxin influx modulators. Plant Physiol. 130:1908-1917.

Saraf M, Pandya U, Thakkar A. 2014. Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. Microbiol Res. 169(1):18-29.

Serrani JC, Ruiz-Rivero O, Fos M, García-Martínez JL. 2008. Auxin-induced fruit-set in tomato is mediated in part by gibberellins. Plant J. 56(6):922-934.

Silva FAZ, Azevedo CAV. 2002. Versão do programa computacional Assistat para o sistema operacional Windows. Rev Bras Prod Agroind. 4(1):71-78.

Spaepen S, Vanderleyden J. 2011. Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol. 3(4):1-13.

Su L, Bassa C, Audran C, Mila I, Cheniclet C, Chevalier C, Bouzayen M, Roustan JP, Chervin, C. 2014. The auxin Sl-IAA17 transcriptional repressor controls fruit size via the regulation of endoreduplication-related cell expansion. Plant Cell Physiol. 55(11): 1969-1976.

Szilagyi-Zecchin VJ, Ikeda AC, Hungria M, Adamoski D, Kava-Cordeiro V, Glienke C, Galli-Terasawa LV. 2014. Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. AMB Express. 4(26):1-9.

Szilagyi-Zecchin VJ, Mógor ÁF, Ruaro L, Röder C. 2015a. Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 em cultura orgânica. Revista de Ciências Agrárias. 38(1):26-33.

Szilagyi-Zecchin, VJ, Klosowski AC, Ikeda AC, Hungria M, Galli-Terasawa LV, Kava-Cordeiro V, Glienke C, Mógor AF. 2015b. Potential inoculant strains of Brazilian endophytic bacteria for maize (*Zea mays* L.) growth promotion. IJAAR. 7(4):128-134.

Szilagyi-Zecchin VJ, Mógor ÁF, Figueiredo GGO. 2016. Microbial Inoculants in Sustainable Agricultural Productivity. Uttar Pradesh: Springer. Chapter 1, Strategies for characterization of agriculturally important bacteria; p. 1-21.

Taiz L, Zeiger E. 2013. Plant physiology. 5nd ed. Sunderland: Sinauer Associates, Inc. Publishers.

Thomas P, Upreti R. 2015. Evaluation of tomato seedling root-associated bacterial endophytes towards organic seedling production. Organic Agriculture. 6(2):89-98.

van Tonder CSM, Combrink NJJ. 2003. The effect of plant-growth regulators on the production of out-of-season greenhouse tomatoes (*Lycopersicum esculentum*). S Afr J Plant & Soil. 20(4):165-168.

Vardharajula S, Zulfikar Ali S, Grover M, Reddy G, Bandi V. 2011. Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact. 6(1):1-14.

Winters AL, Lloyd JD, Jones R, Merry RJ. 2002. Evaluation of a rapid method for estimating free amino acids in silages. Anim Feed Sci Technol. 99(1):177-187.

Xu G, Fan X, Miller AJ. 2012. Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol. 63:153-182.

Zhang H, Kim MS, Krishnamachari V, Payton P, Sun Y, Grimson M, Farag MA, Ryu CM, Allen R, Melo IS, Paré PW. 2007. Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. Planta. 226:839-851.

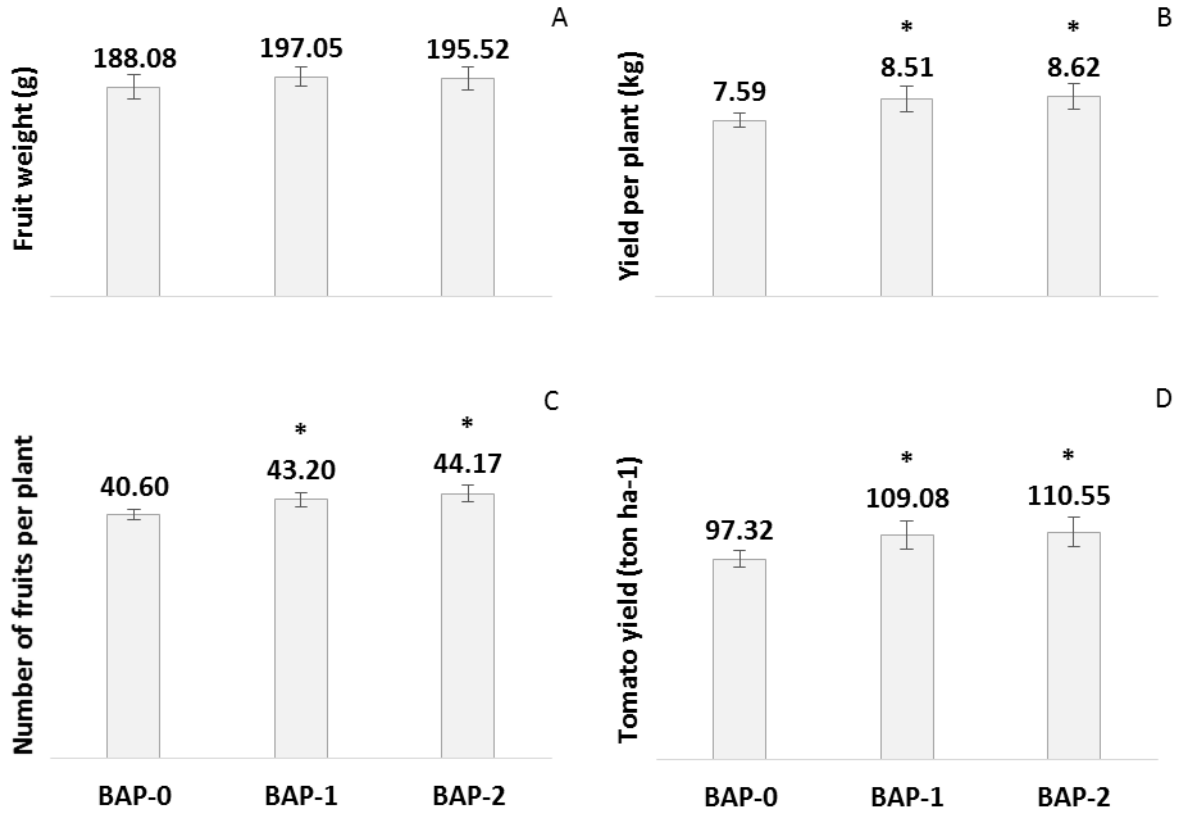

Figure 1. Average values of tomato fruit measurements at the end of the harvest season: (A) fruit weight (g), (B) yield per plant (kg), (C) number of fruits per plant, (D) tomato yield (ton ha^{-1}) considering 12,820 plants ha^{-1}. (*) significant difference by Scott-Knott test $p \leq 0.05$.

.

Table 1. Number of fruits per plant classified by transverse diameter according to commercial rate. (GA) general average, (CV %) coeficiente of variation.

	Transverse diameter of fruits (mm)			
	50-65	65-80	80-100	>100
BAP-0	7.9 a	24.5	7.6 c	0.9
BAP-1	6.7 b	26.1	10.1 a	0.8
BAP-2	6.7 b	25.3	11.1 a	1.1
GA	7.1	25.3	9.6	0.93
CV (%)	9.83	ns	6.91**	ns

Scott-Knott test $p \leq 0.05$, **$p \leq 0.01$, (ns) no significative difference between average.

Table 2. Biochemical analysis of tomato leaves and fuits at 135 days after sowing. (CV%) coefficient of variation. Metabolites expressed in mg g^{-1} of fresh weight.

	Total soluble sugars	Reducing sugars	Total soluble proteins	Total free amino acids
		Leaf		
BAP-0	4.98 c	2.77 b	4.70	0.26 b
BAP-1	5.95 b	3.52 a	4.35	0.39 a
BAP-2	6.40 a	2.57 b	4.61	0.39 a
CV%	4.48**	6.83	ns	18.73**
		Fruit		
BAP-0	31.72	20.29	10.62 b	1.81 b
BAP-1	32.83	20.39	13.39 a	2.03 a
BAP-2	31.10	20.09	13.37 a	2.01 a
CV%	ns	ns	11.03**	6.59

Scott-Knott test $p \leq 0.05$, **$p \leq 0.01$, (ns) no significative difference between average.

Tabela 3. Nutritional analysis of tomato leaves harvested at 135 after sowing. (SNR) Sufficiency Nutrient Ranges, for greenhouse tomato during fruiting (Hochmuth, 2015). (CV%) coeficient of variation %.

	N	Cu	Fe	Zn	Mn
	....%...	.....	ppm	.....	
SNR	3.5-4.0	8.0-20.0	50-200	25-60	50-125
BAP-0	3.19 c	45.99 a	133.87 b	22.20 b	67.13 c
BAP-1	3.43 b	38.50 a	217.13 a	21.95 b	87.45 b
BAP-2	3.77 a	11.55 b	199.88 a	25.07 a	105.98 a
CV%	2.31**	9.31**	13.01**	5.39	7.88**

Scott-Knott test $p \leq 0.05$, **$p \leq 0.01$.

7 CONCLUSÕES GERAIS

A bactéria FZB42 apresentou característica benéficas à promoção de crescimento vegetal por meio da produção de sideróforos e compostos indólicos.

As doses de FZB42 testadas não afetaram negativamente a germinação de sementes e o desenvolvimento inicial das plântulas.

Na produção de mudas as doses $1,5x10^{9}$; $6,0x10^{10}$ UFC mL^{-1} proporcionaram plantas com sistema radicular maior e parte aérea mais desenvolvida. E a dose $2,4x10^{11}$ UFC mL^{-1} atuou de maneira inversa, denotando um efeito inibidor nesse estádio fenológico.

Plantas com cerca de 60 dias tiveram desenvolvimento vegetativo semelhante às mudas, de acordo com as doses aplicadas.

A inoculação promoveu incrementos na produtividade de cerca de 12%, este aumento foi reflexo do maior número de frutos por planta e do calibre do fruto.

FZB42 foi capaz de modular na planta compostos do metabolismo primário como açucares, proteínas e aminoácidos, tanto nas folhas como nos frutos.

Além disso, interferiu no metabolismo secundário por meio de alterações nos teores de clorofilas e carotenoides.

Pelo monitoramento dos níveis nutricionais das plantas, observou-se que FZB42 proporcionou um acúmulo de nitrogênio, ferro, manganês e zinco nas folhas.

Sendo assim, a hipótese formulada no início do estudo foi comprovada com limite de dose, pois FZB42 promoveu o crescimento das plantas de tomateiro, mas não de maneira crescente de acordo com as doses testadas pois a maior dose inibiu o desenvolvimento das plantas em vários estádios fenológicos.

Os genótipos de tomateiro testados mostraram respostas à inoculação com diferentes intensidade, mas todos foram sensíveis a ação bacteriana.

8 CONSIDERAÇÕES FINAIS

Mediante os resultados obtidos no presente trabalho, verificou-se que alguns dados encontrados abrem oportunidades para direcionar futuros trabalhos.

Os resultados apresentados contemplam vários estádios do desenvolvimento do tomateiro, e partem da inoculação da bactéria FZB42 na semente no momento do plantio. Portanto monitorar a colonização da cepa nas raízes e parte aérea, por qPCR e/ou microscopia de fluorescência mediante transformação genética com gene marcador (por exemplo GFP), elucidaria em parte os efeitos encontrados a longo prazo, sendo possível que esta bactéria tenha um modo de vida endofítico.

É de conhecimento que esta cepa produz compostos indólicos e que sua atuação na interação com a planta envolve hormônios vegetais e bacterianos, sendo assim quantificar nas plantas inoculadas alterações hormonais, por HPLC-MS, quantificaria os efeitos auxínicos encontrados.

Os testes bioquímicos foram executados utilizando as folhas, mas de maneira complementar, estes mesmos testes poderiam ser conduzidos usando as raízes a fim de auxiliar nas justificativas das relações de fonte e dreno.

Como a bactéria FZB42 foi capaz de aumentar os níveis de nitrogênio na folha, mesmo não tendo genes para fixação biológica de nitrogênio, cogita-se a hipótese que ela esteja atuando nas rotas de conversão de nitrato a nitrito, portanto medir a atividade da enzima nitrato redutase poderia se útil para elucidar esta hipótese. Ou ainda possa haver uma atuação na sequencia da rota metabólica, especificamente na síntese de aminoácidos tendo em vista o aumento acentuado de aminoácidos nas folhas e frutos.

Seria de grande valia para melhor uso do potencial biotecnológico desta cepa testar seu uso considerando sua capacidade solubilizadora de fosfato inorgânico. Pois a mesma já foi descrtia para esta característica e no presente trabalho, embora não tenhamos relatado essa habilidade, a mesma foi testada e comprovada.

9 REFERÊNCIAS GERAIS

ARAUJO, F. F.; HUNGRIA, M. Nodulação e rendimento de soja co-infectada com *Bacillus subtilis* e *Bradyrhizobium japonicum/B. elkanii*. **Pesquisa agropecuária brasileira**, v. 34, p. 1633-1643, 1999.

BORRISS, R.; CHEN, X.H.; RUECKERT, C.; BLOM, J.; BECKER, A.; BAUMGARTH, B.; FAN, B.; PUKALL, R.; SCHUMANN, P.; SPRÖER, C.; JUNGE, H.; VATER, J.; PÜHLER, A.; KLENK, H.P. Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM7T and FZB42T: a proposal for *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* subsp. nov. and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on complete genome sequence comparisons. **International Journal of Systematic and Evolutionary Microbiology**, v. 61, p. 1786-1801, 2011.

CHEN, X. H.; KOUMOUTSI, A.; SCHOLZ, R.; EISENREICH, A.; SCHNEIDER, K.; HEINEMEYER, I.; MORGENSTERN, B.; VOSS, B.; HESS, W. R.; REVA, O.; JUNGE, H.; VOIGT, B.; JUNGBLUT, P. R.; VATER, J.; SÜSSMUTH, R.; LIESEGANG, H.; STRITTMATTER, A.; GOTTSCHALK, G. E BORRISS, R. Comparative analysis of the complete genome sequence of the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42. **Nature Biotechnology**, v. 25, p. 1007-1014, 2007.

CUARTAS, C. A. R. **Exploring the Relation Between Plant Phosphorus Nutrition and Growth Promotion by *Bacillus subtilis/amyloliquefaciens* Strains.** 72 f. Dissertation (Doctorate on Philosophy) – University of Auburn, Auburn, 2010.

FAO - Food and Agriculture Organization of the United Nations. **Base de dados Faostat – Agriculture, 2013**. Roma: FAO, 2015. Disponível em: < http://faostat.fao.org/site/339/default.aspx>. Acesso em: 20/04/2016.

FREITAS, S. S.; PIZZINATTO, M. A. Ação de rizobactérias sobre a incidência de *Colletotrichum gossypii* e promoção de crescimento em plântulas de algodoeiro (*Gossypium hirsutum*). **Summa Phytopathologica**, v. 23, p. 36-41, 1997.

GARCÍA, J. A. L.; PROBANZA, A.; RAMOS, B.; PALOMINO, M.; MAŅERO, F. J. G. Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. **Agronomie**, v. 24, n. 4, p. 169-176, 2004.

HARTHMANN, O. E. L.; MÓGOR, A. F.; WORDELL FILHO, J. A.; LUZ, W. C. D. Rizobactérias no crescimento e na produtividade da cebola. **Ciência Rural**, v. 40, n. 2, p. 462-465, 2010.

IDRISS, E.E.S.; MAKAREWICZ, O.; FAROUK, A.; ROSNER, K.; GREINER, R.; BOCHOW, H.; RICHTER, T.; BORRISS, R. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB 45 contributes to its plantgrowth-promoting effect. **Microbiology,** v. 148, n. 2097-2109, 2002.

IDRIS, E. E.; IGLESIAS, D. J.; TALON, M.; BORRISS, R. Tryptophan dependente production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. **Molecular Plant-Microbe Interactions Journal**, v. 20, p. 619-626, 2007.

LUCY, M.; REED, E.; GLICK, B.R. Application of free living plant growth-promoting rhizobacteria. **Antonie van Leewenhoek**, v. 86, p. 1-25, 2004.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento. **Decreto 4.954. 2004**. SISLEGIS. Disponível em: <http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do>. Acesso em: 15/04/2012.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa nº46. 2011**. SISLEGIS. Disponível em: <http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do>. Acesso em: 15/04/2012.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa nº13. 2011**. SISLEGIS. Disponível em: <http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do>. Acesso em: 15/04/2012.

PETRAS, S. F.; CASIDA, L. E. J. Survival of *Bacillus thuringiensis* spores in soil. **Applied and Environmental Microbiology**, v. 50, p. 1496-1501, 1985.

STANIER, R.Y., DUODOROFF, M., ADELBERG, E.A **Mundo dos micróbios**. São Paulo: Edgard Blücher, USP, 1969.

SZILAGYI-ZECCHIN, V. J.; IKEDA, A. C.; HUNGRIA, M.; ADAMOSKI, D.; KAVA-CORDEIRO, V.; GLIENKE, C.; GALLI-TERASAWA, L. V. Identification and characterization of endophytic bacteria from corn (*Zea mays* L.) roots with biotechnological potential in agriculture. **AMB Express**, v. 4, n. 1, p. 1-26, 2014.

SZILAGYI-ZECCHIN, V. J.; MÓGOR, Á. F.; RUARO, L.; RÖDER, C. Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 em cultura orgânica. **Revista de Ciências Agrárias**, v. 38, n.1, p. 26-33, 2015a.

SZILAGYI-ZECCHIN, V. J.; KLOSOWSKI, A. C.; IKEDA, A. C.; HUNGRIA, M.; GALLI-TERASAWA, L. V.; KAVA-CORDEIRO, V.; GLIENKE, C.; MÓGOR, A. F. Potential inoculant strains of Brazilian endophytic bacteria for maize (*Zea mays* L.) growth promotion. **International Journal of Agronomy and Agricultural Research**, v. 7, n. 4, p. 128-134, 2015b.

VALE, M.; SELDIN, L.; ARAÚJO, F.F.; LIMA, R. Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari DK (ed) **Plant growth and health promoting bacteria**. Springer, Berlin, p. 21-43, 2010.